The Performance of Serum Cortisol, Plasma Corticotropin and Serum Dehydroepiandrosterone Sulfate Levels in the Diagnosis of Hypothalamic Pituitary Adrenal Dysfunction

ORIGINAL

Abstract

Background: There is no single test can identify hypothalamic-pituitary-adrenal dysfunction (HPAD) perfectly. The study aimed to assess the performance of measuring serum cortisol, serum dehydroepiandrosterone sulfate (DHEA-S) and plasma corticotropin (ACTH) in comparison with standard-dose short cosyntropin (250 µg) testing for the diagnosis of HPAD.

Methods: This is a cross-sectional study from Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC) in Basrah for the period of November 2014 to October 2015. For all patients with suspected HPAD; baseline serum cortisol, serum DHEA-S and plasma ACTH and were measured, followed by a formal short cosyntropin test as the gold standard test.

Results: The total number of the study participant was 169 patients. Of them, 134 (79.3%) were women. Their age ranges from 5-80 years. The cut-off serum cortisol that predicts abnormal short cosyntropin test was less than 5.31 µg/dl with maximal sensitivity and specificity of 87.7% and 90.4% respectively. The cut-off serum DHEA-S that predict abnormal short cosyntropin test was less than 31.11 µg/dl with a sensitivity of 89.2% and specificity of 62.7%. The least reliable parameter to predict the abnormal short cosyntropin was the plasma ACTH level with a cut-off of less than 5.30 pg/ml and lowest sensitivity of 68.8% and specificity of 74.5%.

Conclusion: To exclude HPAD, serum cortisol at 9-11 am having the highest predictive value, DHEA-S has the midway function and plasma

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ACTH is the least reliable for that. Combination of serum cortisol and serum DHEA-S yield the best performance for combinations of test.

Keywords

Hypothalamic Pituitaryadrenal Dysfunction; Short Cosyntropin Test; Dehydroepiandrosterone Sulfate; Corticotropin; Cortisol.

The diagnosis of adrenal insufficiency (AI) is a challenging task, and the lack of awareness of this disease lead to serious consequences, hence untreated AI associated with increase in mortality and morbidity. [1] Adrenocorticotropin (ACTH) hormone handle the function of inner two layers of adrenal gland cortex zona glomerulosa and zona reticularis that secretes glucocorticoid and adrenal androgens respectively. [2] Corticotrophin releasing hormone (CRH) is a peptide hormone secreted from hypothalamus, responsible for secretion of ACTH from pituitary gland. [3] Based on that measurement of plasma ACTH is helpful to differentiate primary and secondary AI. Cortisol and ACTH follow circadian rhythm, cortisol peak in the early morning from 6-8 am. [4] However; early morning basal cortisol used in clinical practice to diagnose AI. Serum cortisol level below 1.8 µg/dl or above 18 µg/dl confirm and exclude AI respectively. [5]

Adrenal androgens androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulphate (DHEA-S) in particular DHEA-S less affected by circadian rhythm and has a long half-life reach 10-20 hours. [6] Normal age and sex specific DHEA-S has sensitivity of 100 % to exclude HPAD. [7] Plasma ACTH less commonly use in clinical practice to diagnose secondary AI because it is normal or lag behind suppression of cortisol and DHEA-S. [8] Dynamic tests remain the best way to diagnose endocrine diseases. In AI, insulin tolerance test or called insulin induced hypoglycemia is gold standard

because of unsafe it is prohibited now a days. [9] The most common and currently used to evaluate Hypothalamic-pituitary-adrenal (HPA) axis dysfunction is short cosyntropin test, it has a shortcomings in the performance that stand clearly from specificity and sensitivity analysis which is as low as 57%-79%. [10-11] Searching for other methods to diagnose HPA integrity that have high specificity and sensitivity are needed.

The Aim of the study

Was to assess the performance of measuring serum cortisol, serum DHEA-S and plasma ACTH in comparison with standard-dose short cosyntropin (250 µg) testing for diagnosis of HPAD.

Patients and methods**Setting, and Participants**

Participants in this cross-sectional study were the patients selected from Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC), in Basrah for the period of November 2014 to October 2015.

Subject Inclusionary criteria included ambulatory patients with a suspicious diagnosis with HPAD.

Inclusion criteria

All patients with suspicion of secondary AI, in particular, those patients with exogenous steroid exposure.

Exclusion Criteria

- Patients referred from Intensive Care Unit (ICU).
- Currently on glucocorticosteroid therapy replacement in the preceding one week.
- Pregnant women, and those who use an oral contraceptive pills or hormone replacement therapy in the preceding six weeks.
- Those on drugs affect cortisol metabolisms like spironolactone, and antiepileptic drugs.
- Primary AI (Addison's disease).

All subjects provided verbal informed consent, and the ethical committee of the Basrah College of Medicine approved the research protocol.

Principle of the Biochemical tests

All patients given instructions one day before the procedure and fasting state was not necessary. The procedure time was from 9:00-11:00 am. From all patients 10 ml of blood withdrawn for baseline cortisol, ACTH and DHEA-S. All blood samples used were serum except ACTH; it is measured in plasma. This is followed by intramuscular injection of 250- μ g cosyntropin, and cortisol levels measured at 0.30, and 60 minutes. The normal adrenal reserve response is a cortisol value greater than 20 μ g/dL (555 nmol/L) at any time 30, or 60 minutes or both.

Research Instruments

The plasma ACTH, serumcortisol, serum DHEA-S, were measured using electrochemiluminescence immunoassay (ECLA) by cobas e 411 (Roche Diagnostics, Germany) analyzer using manufacturer-supplied reagents and instructions.

For ACTH, the measuring range was 1.0-2000 pg/ml and specified the intra-assay precision of 5-100 pg/ml ($< 5\%$ CV). While cortisol, the measuring range was 0.018-63.4 μ g/dl and specified the intra-assayprecision of > 110 nmol/l ($< 6\%$ CV).

DHEA-S measuring range was 0.100-1000 μ g/dl and specified the intra-assay precision of 50-1000 μ g/dl ($< 5\%$ CV).

Normal reference interval

Plasma ACTH ranges from 7-66 pg/ml according to kit value.

DHEA-S, serum according to Mayo Clinic medical laboratories, reference interval according to age and gender. [12]

Statistical analysis

Data analysis was done using SPSS 15 (SPSS Inc, Chicago, IL, USA). Continuous variables plotted as the mean (SEM). Categorical variables plotted as a percentage.

The area under the receiver-operating characteristics (ROC) curves used to assess the diagnostic performance of each of the three parameters; plasma ACTH Level, serum DHEA-S, and serum cortisol together with combinations of parameters of abnormal cortisol and ACTH (**Panel I**), abnormal DHEA-S and ACTH (**Panel II**), abnormal cortisol and DHEA-S (**Panel III**), and abnormal cortisol, DHEA-S and ACTH (**Panel IV**) to predict the abnormal short cosyntropin test.

The cut-off plasma ACTH Level, serum DHEA-S, and serum cortisol value was calculated by plotting-the true positive rate (sensitivity) against the false positive rate (1-specificity).

Results

The cause of HPAD in our patients was the exogenous use of glucocorticosteroid in almost most of the patients or with symptoms of adrenal dysfunction without a history of drug use. None of the our study sample,were having a pituitary tumor, surgery, or radiation before.

Total number of the study participants was 169 patients (**Table 1**) of them 134 (79.3%) were women. Their ages range from 5-80 years. The majority, 131 patients (77.5%) were in the age range 21-50 years, the mean age is mean $\pm$ SEM age 38.8 $\pm$ 0.94 years. Forty point eight percent were having suppressed ACTH, and 59.2% had a level

Table 1. Patients' characteristics.

		No (%)
Male		35 (20.7)
Female		134 (79.3)
Age(year) range 21-50 (Mean±SEM)		38.9±0.94
DHEA-S	Normal	71 (42.0)
	Low	96 (56.8)
	Missing	2 (1.2)
Cortisol	Normal	102 (60.3)
	Low	67 (39.7)
ACTH	Normal	69 (40.8)
	Low	100 (59.2)
Short cosyntropin test	Normal	104 (61.5)
	Abnormal	65 (38.5)

Table 2. Sensitivity and specificity of plasma ACTH of the short cosyntropin test.

Short cosyntropin test					
		Abnormal Response	Normal response		
Plasma ACTH Level	Low	43	26	62.9%	Positive predictive value
	Normal	22	78	79.2%	Negative predictive value
		68.8%	74.5%		
		Sensitivity	Specificity		
Total		65	104		

Table 3. Sensitivity and specificity of serum DHEA-S to short cosyntropin test.

Short cosyntropin test					
		Abnormal Response	Normal response		
Serum DHEA-S Level	Low	58	38	60.4%	Positive predictive value
	Normal	7	64	90.1%	Negative predictive value
		89.2%	62.7%		
		Sensitivity	Specificity		
Total		65	102		

in the reference range. The mean $\pm$ SEM for ACTH level was 28.6 ± 11.8 pg/ml. Mean $\pm$ SEM of DHEA-S was 75.36 ± 8.6 μ g/dl; it was low in 56.8%. For basal serum cortisol, the mean $\pm$ SEM was 9.7 ± 0.857 μ g/dl. It was low in 39.7%. Results of the short cosyntropin test were abnormal in 56 patients (38.5%).

The sensitivity, specificity, positive predictive values and negative predictive values of all parameters (plasma ACTH Level, serum DHEA-S and serum cortisol) alone in comparison with the standard test, the short cosyntropin test are presented in **(Table 2)**.

The sensitivity of plasma ACTH Level was 68.8%, specificity 74.5%, positive predictive values 62.9% and negative predictive value 79.2%. This mean that the false positive rate was 25.5%, a false negative rate 31.2%, positive likelihood ratio equal to 2.92 and negative likelihood ratio equal to 0.418.

The sensitivity of serum DHEA-S was 89.2%, specificity 62.7%, positive predictive values 60.4% and negative predictive value 90.1%. This means that the false positive rate was 37.3%, a false negative rate 10.8%, positive likelihood ratio equal to 2.39 and negative likelihood ratio equal to 0.172. **(Table 3)**

Level of serum cortisol **(Table 4)** **(Figure 1)** had a sensitivity of 87.7%, specificity 90.4%, positive predictive values 85.1% and negative predictive value

Table 4. Sensitivity and specificity of serum cortisol of short cosyntropin test.

Short cosyntropin test					
		Abnormal Response	Normal response		
Serum Cortisol Level	Low	57	10	85.1%	Positive predictive value
	Normal	8	94	92.2%	Negative predictive value
		87.7%	90.4%		
		Sensitivity	Specificity		
Total		65	104		

Figure 1: Sensitivity, specificity, positive and negative predictive values of different tests compared to short cosyntropin test. **Table 5.** Cut-off values of serum cortisol, DHEA-S and plasma ACTH and their predictive performance that indicate abnormal short.

	The area under ROC curve (95% CI)	Cut-off	Sensitivity	Specificity
Plasma ACTH	0.762 (0.679-0.834)	5.30 pg/ml	68.8%	74.5%
Serum DHEA-S	0.887 (0.829-0.936)	31.11 µg/dl	89.2%	62.7%
Serum cortisol	0.941 (0.903-0.979)	5.31 µg/dl	87.7%	90.4%
Abnormal Cortisol and ACTH (Panel I)	0.775 (0.696-0.855)	-	60.0%	95.1%
Abnormal DHEA-S and ACTH (Panel II)	0.776 (0.697-0.855)	-	63.1%	92.2%
Abnormal cortisol and DHEA-S (Panel III)	0.916 (0.863-0.969)	-	86.2%	97.1%
Abnormal cortisol, DHEA-S and ACTH (Panel IV)	0.795 (0.717-0.873)	-	60.0%	99.0%

92.2%. This means that the false positive rate was 9.6%, a false negative rate 12.3%, positive likelihood ratio equal to 9.13 and negative likelihood ratio equal to 0.136.

The cut-off point of serum cortisol that predicts abnormal short cosyntropin test was less than 5.31 µg/dl of maximal sensitivity and specificity of 87.7% and 90.4% respectively (**Table 5**). The cut-off point of serum DHEA-S that predict abnormal short cosyntropin test was less than 31.11 µg/dl with a sensitivity of 89.2% and specificity of 62.7%. The least reliable parameter to predict the abnormal short cosyntropin was the plasma ACTH level with a cut-off of less than 5.30 pg/ml and lowest sensitivity of 68.8% and specificity of 74.5%.

Performance of baseline parameters in comparison with cosyntropin test results is presented in **Table 5**. Highest Specificity and sensitivity for HPAD were seen with dual basal morning serum cortisol, and serum DHEA-S were it reaches for 86.2% and 97.1%, respectively. The only comparable to that was basal morning cortisol with specificity and sensitivity of 87.7% and 90.4%, respectively. The combination of abnormal all parameters of serum cortisol, serum DHEA-S, and plasma ACTH will improve the specificity to 99%, but reduced the sensitivity to very low level (60.0%).

The use of a panel of tests combination to improve the performance of predicting abnormal short cosyntropin test yield that, a combination of morning serum cortisol and serum DHEA-S will have the best performance.

Discussion

Glucocorticosteroids nonprescription use is not rare in Iraq for years. This makes the risk of HPAD prevalence very high, especially with denial of use. [13]

The availability of short cosyntropin test can not guarantee always, and the insulin stress test is dangerous in our locality. [14]

For that reason, we tried to have a panel of simple tests which are feasible and available to assess the risk of HPAD, which can be fatal if missed. [15-16]

A majority of HPAD seen in our Center is due to use of exogenous corticosteroids for various reasons, but most of them are due to nonprescription use. [13] This is established fact, all over the world. [16]

Adrenal insufficiency after discontinuation of glucocorticoid occurs frequently; there is no administration form, dosing, treatment duration, or underlying disease for which adrenal insufficiency it can be excluded with certainty. [17]

More than two third of patients in this study were women in the age range 21-50. Similar finding seen from the same Center before, where women constituted 69% of those self-prescribed corticosteroids. [13] In a study by Hahner et al., women to men ratio for secondary AI were 113:77 with median age of 59 years. [18] The female predominance for HPAD is due to more use of corticosteroids in our patients that difficult to explain.

The best performance seen in this study was for serum cortisol from 9-11 am with the morning serum cortisol of $<5.31 \mu\text{g/dl}$ goes with the diagnosis of HPAD. Others advocate that serum cortisol $\leq 5 \mu\text{g/dL}$ to confirm HPAD. [5, 10-11, 19]

In children some suggested that un-stimulated serum cortisol $<3.9 \mu\text{g/dL}$ is mandatory for the diagnosis of HPAD, if used alone with morning cortisol $>13.8 \mu\text{g/dl}$ goes with normal function and they advised doing dynamic stimulation tests only for those with morning cortisol between $3.9-13.8 \mu\text{g/dl}$. [20]

The performance of DHEA-S was medium in between. One study found that normal age and gender-specific value of DHEA-S exclude HPAD in 100 %. [6] It's had been found that serum DHEA-S fall even before the serum cortisol in those with a history of exogenous use of glucocorticosteroid and lasted up to one year after drug stoppage, which is the case in our study. [6]

Serum DHEA-S used as screening test to diagnose HPAD are advocated. [16] However; DHEA-S if not enough for diagnosis, and low values need to be confirmed by other tests for adrenal function. [5] DHEA-S could not differentiate between primary or secondary adrenal failure and low DHEA-S also found after post-traumatic stress disorder and depressive symptoms. [21]

The worst performance was plasma ACTH in this study. This result proved before, where it is useful in the diagnosis of primary AI. [17] This can be explained on the basis, that cortisol derangement in HPAD could be due to an ACTH-independent mechanism, one of which reduce exertion and metabolism of cortisol by the liver and kidney that coined the term relative insufficiency of the hypothalamic-pituitary-adrenal axis. [22-25] These mechanisms could increase cortisol during stress and save human life, but stress last for more than a week would affect the affect the adrenocortical integrity and function. [26]

Furthermore, reduced response to exogenous corticotropin could be due to reduce adrenocortical ACTH signaling that affects the results of dynamic tests to diagnose the HPAD. That's why the short cosyntropin test carries poor sensitivity, which can be a wide range (57%-79%). [17] Nevertheless, the normal short cosyntropin test confirms more normality of the HPA axis. [27]

The HPAD is usually characteristically partial due to partial secretion of CRH and or ACTH. [11] For that reason, the diagnosis of the HPAD is quite challenging for the most endocrinologist. Patients with partial nature of ACTH deficiency will have a no-load response to short cosyntropin test, especially if the cut-off point of cortisol will be $18.1 \mu\text{g/dl}$ and below, so cut-off of $20 \mu\text{g/dl}$ will improve the sensitivity of the test. [6]

The use of a panel of two tests to predict HPAD was explored in this study where the combination of morning serum cortisol and serum DHEA-S gave the best results to predict dysfunction. This was esta-

blished recommendation by some endocrinologist. [5] The use of three panels of tests involving serum cortisol, serum DHEA-S, and plasma ACTH will have the best specificity, but very poor sensitivity. This finding is not explored before. [5]

The potential application of this study is that DHEA-S used as a tool to diagnose hypothalamic pituitary adrenal dysfunction in the afternoon because DHEA-S not subjected to circadian rhythm due to prolonged half-life. [11, 20, 21, 26-28] Since the majority of HPAD will present at afternoon to the emergency units or hospitals were the short cosyntropin not feasible and serum cortisol, and plasma ACTH will become unreliable.

Study limitation

the gold standard test in this study was a short cosyntropin test and not the insulin stress test. Because the insulin stress test carries many dangers. [15, 29] And, DHEA-S cut-off we used was regardless the age and gender. Furthermore, measurements of serum DHEA levels during the short cosyntropin test would add more benefit in the interpretation of short cosyntropin test with the improvement of diagnostic accuracy. [8]

Conclusion

To exclude HPAD, serum cortisol at 9-11 have the highest predictive value, DHEA-s has the midway function, and plasma ACTH is the least reliable for that. The combination of serum cortisol and serum DHEA-s yield the best performance for combinations of the combinations of the test

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