

Evaluation of Hypothalamo-Pituitary-Adrenal Axis Activity by Using Dexamethasone Suppression Test in Patients with Panic Disorder and Generalized Anxiety Disorder

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ABSTRACT

Evaluation of hypothalamo-pituitary-adrenal axis activity by using dexamethasone suppression test in patients with panic disorder and generalized anxiety disorder

Objective: The aim of this study was to investigate the role of hypothalamo-pituitary-adrenal (HPA) axis activity on the pathophysiology of anxiety disorders by examining the HPA axis activity in patients with panic disorder (PD) and generalized anxiety disorder (GAD).

Method: Baseline and post dexamethasone suppression test (DST) serum concentrations of cortisol and dehydroepiandrosterone-sulfate (DHEA-S) were measured in patients with PD (n=24), GAD (n=21) and in healthy controls (n=20).

Results: The baseline cortisol levels in GAD group were found lower than those of the patients with PD and healthy controls. Cortisol suppression by dexamethasone in GAD group was found to be lower than PD patients and healthy controls. Baseline and post-DST DHEA-S levels in the patients with PD and GAD were similar to those of the healthy controls.

Conclusion: Lower cortisol levels and inadequate cortisol suppression with DST in GAD patients may suggest a downregulation in both corticotropin releasing hormone and glucocorticoid receptors in the central nervous system.

Keywords: Dehydroepiandrosterone, dexamethasone, generalized anxiety disorder, panic disorder



ÖZET

Panik bozukluğu ve yaygın anksiyete bozukluğu olan hastalarda hipotalamo-pituitar-adrenal eksen aktivitesinin deksametazon baskılama testi ile değerlendirilmesi

Amaç: Bu çalışmanın amacı panik bozukluğu (PB) ve yaygın anksiyete bozukluğu (YAB) olan hastalarda hipotalamo-pituitar-adrenal (HPA) eksen aktivitesini inceleyerek, bu sistemin PB ve YAB'nin patofizyolojisindeki rolünü araştırmaktır.

Yöntem: PB (n=24) ve YAB (n=21) olan hastalarda ve sağlıklı kontrollerde (n=20) bazal ve deksametazon baskılama testi (DST) uygulaması sonrasında kortizol ve dehidroepiandrosteron-sülfat (DHEA-S) değerleri ölçüldü.

Bulgular: YAB grubunun bazal kortizol düzeyleri kontrol ve PB grubuna göre düşük bulundu. Ayrıca YAB grubunda deksametazonun kortizolu baskılaması kontrol ve PB grubuna göre düşük bulundu. PB ve YAB hastalarında bazal ve DST sonu DHEA-S düzeyleri kontrol grubuna benzer bulundu.

Sonuç: YAB hastalarında bazal kortizol düzeyinin düşük olması ve DST'de kortizol baskılanmasının yetersiz oluşu bu hastalarda hem kortikotropin salgılatıcı hormon reseptörlerinde, hem de glukokortikoid reseptörlerinde bir aşağı ayarlama (down-regulation) olduğunu akla getirmektedir.

Anahtar kelimeler: Dehidroepiandrosteron, deksametazon, yaygın anksiyete bozukluğu, panik bozukluğu

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INTRODUCTION

Anxiety disorders are the most common mental disorders. Panic disorder (PD) and generalized anxiety disorder (GAD) constitute an important part of anxiety disorders (1). Research on neurobiology of PD and GAD has focused on hypothalamic-pituitary-adrenal (HPA) axis—especially on cortisol—as well as provocation studies. HPA axis activity in PD has been evaluated by cortisol levels in the, urine, saliva and plasma; cortisol response to dexamethasone suppression test (DST); and Adrenocorticotrophic hormone (ACTH) and cortisol response to dexamethasone-corticotropin-releasing hormone (CRH) test (2,3). However studies on HPA axis activity in GAD are few. In patients with GAD, DST positivity has been reported at approximately 27% (4) and 38% (5). Although this rate is less than depression patients, it indicates that there is also an abnormal response to stress in GAD patients and there is a defect in regulatory feedback mechanisms of HPA axis. There are reports stating that cortisol levels in GAD patients are low (6,7), normal (8) and-in elderly patients-high (9,10). In conclusion, findings on HPA axis abnormalities in PD and GAD appear to be conflicting and inadequate (11).

There is also evidence that other steroid hormones secreted from the adrenal gland may also be etiologically related to anxiety disorders (12,13). Dehydroepiandrosterone (DHEA) and sulphate ester of DHEA, dehydroepiandrosterone-sulfate (DHEA-S), are among these hormones. DHEA-S is mainly derived from the adrenal gland, and is also synthesized as 'de novo' in the brain. The highly correlated cerebrospinal fluid and serum concentrations of DHEA-S suggests that the serum level measurement will be an indicative of brain level (14). DHEA shows anti-stress properties (15). Low levels of DHEA and DHEA-S are associated with high levels of perceived stress and trait anxiety (16). DHEA increases and acts as a marker in response to acute stress. DHEA-S exhibits antiglucocorticoid property by suppressing cortisol (16). Although it is known that it is largely secreted from the adrenal gland, the factors that play a role in the regulation of DHEA-S are not clear.

ACTH is considered to stimulate the production of DHEA from adrenals, though it is known that there are some differences between cortisol and DHEA release in certain situations (14). Dexamethasone may be suppressing DHEA-S through other mechanisms independent of ACTH. This suppression can be evaluated by checking the DHEA-S response to DST. There are few studies on DHEA-S in PD and GAD (2). In PD cases, there are reports indicating normal (17), as well as high baseline DHEA (18) levels. In a study, GAD patients have showed lower levels of pregnanolon sulphate than the control group, but no difference in DHEA-S levels (12).

In this study, it was aimed to investigate possible HPA axis defects in patients with PD and GAD, by examining baseline cortisol, baseline DHEA-S; and cortisol and DHEA-S responses to dexamethasone.

METHOD

This is a non-blind clinical experimental research study. Patients between the ages of 20 and 48 years who were admitted to the psychiatry outpatient clinic and diagnosed with PD or GAD according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Revised Fourth Edition (DSM-IV TR) (19) were included in the study as the case group, and healthy volunteers as the control group. The diagnosis was made by two psychiatrists independently.

Patients with concurrent or history of any other psychiatric disorder; any neurological disease which may cause organic brain disorders such as head trauma, epilepsy, alcohol or substance abuse; endocrinological or metabolic diseases; women receiving estrogen replacement therapy during the study; and those who have had electroconvulsive therapy in the last 6 months were excluded. Twenty-four PD patients (mean age 38.4 ± 7.2 years, 10 females, 14 males) and 21 GAD patients (mean age 30.7 ± 8.8 years, 13 females, 8 males) that met the criteria, were included in the study. The control group consisted of 20 healthy individuals (mean age 30.0 ± 4.4 years, 10 females, 10 males) who met the same criteria and who

did not have a present or past history of any psychiatric disorder (Table 1). Patient and control group were hospitalized for 3 days throughout the study. Patients and controls were elected by performing their physical, psychiatric and neurological examinations, routine biochemical tests, whole blood counts, and thyroid function tests. The research protocol was approved by the Ethics Committee of Erciyes University Faculty of Medicine. Patients and controls were informed about the objectives and the design of the study and written consents were obtained.

Data Collection Tools

Nine of the 24 PD patients and 8 of the 21 GAD patients were on medication. Medications were stopped and they were included in the study at the end of the two-week out of the hospital drug clearance period. The Hamilton Depression Rating Scale (HDRS) (20) was used to assess depression levels, and the Clinical Anxiety Scale (CAS) (21) was used to assess anxiety levels in patients and controls. Those with a HDRS score lower than 7 were included in the study.

As performed in similar suppression studies (22), after one night fasting, blood samples were taken at 09.00 a.m. by a catheter placed in the antecubital vein to measure baseline cortisol and DHEA-S levels from the patient and control groups. Immediately after blood samples were taken, 0.5mg dexamethasone was administered orally for two days at 09.00, 15.00, 21.00 and 03.00 o'clock. On the third day at 09.00 a.m. blood samples were taken once again for cortisol and DHEA-S levels. Blood samples were centrifuged (4000rpm) for 5 minutes within two hours of drawing and then the sera were stored at -70°C until analyzed.

Biochemical Analysis

Serum cortisol and DHEA-S level measurements were made by radioimmunoassay (RIA). Serum cortisol level was measured with RIA (DSL-2100) kit. Its sensitivity limit was 0.3µg/dL, the intra- and inter-assay coefficients of variation were 5.3% at 19.21µg/dL

concentration and 8.9% at 19.18µg/dL concentration, respectively. Serum DHEA-S level was measured with RIA (DSL-3500) kit. The sensitivity of this assay was 1.7µg/dL, the intra- and inter-assay coefficients of variation were 7.8% at 14.5µg/dL concentration and 10.0% at 17.3µg/dL concentration, respectively.

Statistical Analyses

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, IBM Inc., USA) 22.0 package program. To assess the response of hormones to DST, cortisol level and change in DHEA-S response (Δ values) after dexamethasone use were calculated.

Δ cortisol: (post-DST cortisol level) - (baseline cortisol level)

Δ DHEA-S: (post-DST DHEA-S value) - (baseline DHEA-S value)

The Kolmogorov-Smirnov test was used to assess normality of distribution for all of the continuous variables. MANOVA with posthoc Bonferroni test were used to assess the difference in terms of age, body mass index (BMI), HDRS and CAS scores between PD, YAD, and control groups. The Mann-Whitney U-test was used to compare the disease duration of the PD and YAD groups. The Kruskal-Wallis H test was used to compare the number of cigarettes smoked per day, because of not normally distribution. The gender distribution difference between genders PD, YAD, and control group was assessed by chi-square (χ^2) test.

MANCOVA test was used to compare baseline cortisol, post-DST cortisol, Δ cortisol, baseline DHEA-S, post-DST DHEA-S, Δ DHEA-S values between PD, YAD and control groups. Gender, age, BMI, and the number of cigarettes smoked daily were used as the covariates. When there was a statistically significant difference between the groups, the difference between the given two groups was determined using the post-hoc Bonferroni test. Pearson correlation coefficients were calculated to evaluate the relationship between demographic data and hormonal values of the patients. The statistical significance level was considered as $p < 0.05$ in this study.

RESULTS

Sociodemographic Characteristics of Groups

There was no significant difference between groups in terms of gender distribution ($\chi^2=1.50$; $df=2$; $p>0.05$). Statistically significant differences were found between PD, GAD and control groups when age, BMI, HDRS, and CAS scores were compared with MANOVA ($F=10.46$; $df=2.63$; $p=0.001$, $F=4.71$; $df=2.63$; $p=0.01$, $F=240.31$; $df=2.63$; $p=0.001$ and $F=277.38$; $df=2.63$; $p=0.001$). Binary group differences were determined by post-hoc Bonferroni tests. The PD group's mean age was greater than the control and GAD groups. BMI was higher in the PD group than in the control group. There was no significant difference between PD and GAD in terms of clinical scales but both groups had higher HDRS and CAS scores than the control group. The duration of disease of the GAD

group was significantly longer than the PD group ($z=-2.75$, $p=0.006$). Smoking rate was 55.0% for the control group, 36.0% for the PD group and 52.2% for the GAD group and there was no statistical difference between them ($\chi^2=1.68$; $df=2$; $p>0.05$). There was no significant difference between the groups in terms of number of cigarettes smoked daily ($\chi^2=2.67$; $df=2$; $p>0.05$) (Table 1).

Baseline and Post-DST Cortisol Levels

There was no statistically significant difference in baseline cortisol levels between the control and PD groups ($F=5.10$; $df=2.58$; $p>0.05$). The baseline cortisol level of the GAD group was significantly lower than the control group and the PD group ($F=5.10$; $df=2.58$; $p=0.009$) (Table 2). There was no significant difference between the groups in terms of post-DST cortisol levels ($F=0.95$; $df=2.58$; $p>0.05$).

Table 1: The sociodemographic and clinical characteristics of groups

	Control (n=20)		PD (n=24)		GAD (n=21)		F	df	p
	Mean	SD	Mean	SD	Mean	SD			
Age (years)	30.00	4.40	38.40*	7.20	30.70	8.80	10.46	2.63	<0.001
BMI (kg/m ²)	23.20	3.60	27.20**	4.60	25.10	4.70	4.71	2.63	<0.01
HDRS	2.20	0.52	6.33**	0.70	6.43**	0.84	240.31	2.63	<0.001
CAS	1.95	0.75	14.45**	1.84	13.65**	2.56	277.38	2.63	<0.001
	Median (25-75%)		Median (25-75%)		Median (25-75%)		χ^2	df	p
Cigarette (n/day)	20 (10.0/20.0)		20 (16.25/21.0)		17.5 (8.75/20.0)		2.67 ^a	2.00	>0.05
Duration of the disease (month)	----		12 (6-27)		36 (21-60)***		$z=-2.75$		0.006
Gender (male/female)	10/10		10/14		13/8		1.50	2.00	>0.05
Smoking (%)	55.0		36.0		52.2		1.68	2.00	>0.05

n: number, SD: Standard Deviation, PD: Panic Disorder, GAD: Generalized Anxiety Disorder, BMI: Body Mass Index, CAS: Clinical Anxiety Scale, HDRS: Hamilton Depression Rating Scale, F: MANOVA, χ^2 : Chi-square, z: Mann-Whitney U, a: Kruskal-Wallis H, *Higher than control and GAD groups, **Higher than control group, ***Higher than PD group

Table 2: Hormonal data of patient and control groups

	Control (n=20)		PD (n=24)		GAD (n=21)		F	df	p
	Mean	SD	Mean	SD	Mean	SD			
Baseline cortisol (µg/dL)	21.52	6.73	19.87	9.66	14.07*	5.64	5.10	2.58	0.009
Post-DST cortisol	2.08	0.84	1.72	0.92	1.58	1.15	0.95	2.58	>0.05
Δ cortisol	-18.08	8.13	-18.14	9.44	-12.48*	5.21	4.00	2.58	0.024
Baseline DHEA-S (µg/dL)	381.66	241.03	290.20	158.97	252.80	190.74	1.45	2.58	>0.05
Post-DST DHEA-S	140.20	99.55	111.41	79.69	100.33	100.48	0.38	2.58	>0.05
Δ DHEA-S	241.45	189.71	-178.79	103.88	-152.47	112.82	1.59	2.58	>0.05

n: number of subjects, SD: Standard Deviation, PD: Panic Disorder, GAD: Generalized Anxiety Disorder, DST: dexamethasone suppression test, F: MANCOVA, DHEA-S: dehydroepiandrosterone-sulfate, Δ cortisol: (post-DST cortisol value) – (baseline cortisol value), Δ DHEA-S: (post-DST DHEA-S value) – (baseline DHEA-S value), *Lower than control and PD groups

There was no significant difference between PD and control group in terms of Δ cortisol (post-DST cortisol value - baseline cortisol value) ($F=4.00$; $df=2.58$; $p>0.05$). However, Δ cortisol was significantly lower in the GAD group than in the PD and control group ($F=4.00$; $df=2.58$; $p=0.024$) (Table 2). In other words, cortisol was less suppressed by dexamethasone in the GAD group than in the PD and control group.

Baseline and Post-DST DHEA-S Values

There was no statistically significant difference between groups in baseline and post-DST DHEA-S levels ($F=1.45$; $df=2.58$; $p>0.05$, $F=0.38$; $df=2.58$; $p>0.05$). There was no significant difference between the groups in terms of Δ DHEA-S (post-DST DHEA-S value - baseline DHEA-S value) ($F=1.59$; $df=2.58$; $p>0.05$) (Table 2).

Correlation Studies in Patient Groups

In the PD group:

As the CAS scores increased, baseline cortisol levels decreased ($r=-0.45$; $p=0.012$). As the CAS scores increased, the Δ cortisol increased ($r=0.45$; $p<0.01$). That is, as the severity of anxiety increased, dexamethasone suppressed cortisol less.

In the GAD group:

There was no significant correlation between baseline cortisol levels and CAS scores ($r=-0.24$; $p>0.05$). As the CAS scores increased, post-DST cortisol level decreased ($r=-0.66$; $p<0.01$).

DISCUSSION

There are studies indicating normal (23-26) as well as increased (27-30) baseline cortisol levels in PD. Our findings were consistent with the studies (23-26) which have found normal cortisol levels in PD. Ninety-five percent of plasma cortisol binds to proteins. The active part is free cortisol. Therefore, one reason for the differences may be that some

studies may have measured free cortisol levels whereas others have measured total cortisol values. Another cause of inconsistency in the results may be the difference in severity of associated depressive symptoms (28) and anxiety levels (31). Another reason why the results are inconsistent may be that the measurement times are different. It has been suggested that some patients may have a change in the chronobiological rhythm of cortisol in the presence of a phase delay (24). In this study blood samples were taken in the morning, and the patients had no associated depression. Total cortisol was measured in the study. Measuring free cortisol could provide a more precise assessment.

In this study, cortisol response to dexamethasone in PD patients was similar to controls. There are studies showing that in PD dexamethasone suppressed cortisol similar to controls (3,23,32) and that not suppressed up to 27% (5). The findings of this study are consistent with studies that dexamethasone cortisol suppression in PD was normal (3,23,32). In PD, the rate of suppression of cortisol with DST is generally low compared to depression. In addition, the fact that in some previous studies, depression has not been completely excluded (28), the presence of associated agoraphobia, and the difference in the threshold values of cortisol (5) may be other causes of nonsuppression. In this study, besides the patients having depression, patients who had a history of depression were also excluded.

In our study, baseline cortisol levels in the GAD group were lower than in the PD and control group. Some of the previous studies on GAD reported baseline cortisol levels similar to the control group (8,33) and higher in the elderly patients than in the control group (10,34). However, in the study by Steudte et al. (7), cortisol levels were found to be 50-60% lower in the GAD patients, compared to the control group in hair analysis that reflected cortisol release retrospectively for up to 6 months. Cortisol has diurnal variation. Cortisol release increases after wake up in the morning, which is called the cortisol awakening response. This response has been found to be lower in GAD patients than in healthy controls (6).

The results of our study are consistent with the studies in which cortisol has been found low in GAD (6,7).

Low cortisol finding in the study of Steudte et al. (7) has been interpreted as a result of the long-term compensatory effort against the initial high cortisol level seen in the GAD. Another explanation is that, initial low levels of cortisol caused anxiety awareness which triggered the emergence of anxiety chains, thus leading to the formation of GAD (7). Among the anxiety disorders, low cortisol is often a finding in posttraumatic stress disorder (PTSD) (35,36), which is explained by increased sensitivity to feedback inhibition by cortisol in the HPA axis (36). In addition to a reduction in the corticosteroid-binding globulines in these patients, there may also be a reduction in hypophyseal and adrenal sensitivity. In summary, at this point it is difficult to say whether low level of cortisol found in anxiety disorders is a consequence or a predisposing factor of the disease.

In addition to posttraumatic stress disorder, hypocortisolemia is a common finding in stress-related disorders such as fibromyalgia and chronic fatigue immune dysfunction syndrome (CFIDS) (37,38). Suggested possible causes of hypocortisolemia include: 1) A decrease in biosynthesis at various levels of the HPA axis or a decrease in secretory factors (eg decrease in release of CRH from hypothalamus, ACTH from pituitary and cortisol from adrenal glands), 2) Downregulation of target receptors following an overrelease of a secretory factor, 3) Oversensitivity in negative feedback of glucocorticoids 4) A decrease in the formation of free cortisol; 5) A decrease in cortisol peripheral depletion due to decreased degradation enzyme activities and thus a decrease in the baseline activity of the HPA axis since less cortisol will be needed (37,38).

It is known that early life stressors predispose to some anxiety disorders such as PTSD and GAD (39). It has been reported that up to 94.8% of GAD patients were exposed to any trauma (40). GAD may resemble PTSD in this respect and the mechanism of hypocortisolemia may be similar. In conditions associated with severe trauma, such as PTSD and

CFIDS, it is supposed that usually there is an initial extreme increase in CRH, resulting in downregulation of CRH receptors and HPA axis hypoactivity (41,42). Our finding of hypocortisolemia in the GAD may be related to their past history of trauma. However, this opinion remains speculative because in this study we had not questioned whether or not the patients had experienced trauma in the past.

In this study, dexamethasone's cortisol suppression in GAD was less than in the control and PD group. In some studies on GAD, dexamethasone did not suppress cortisol at rates of 27% (4,43) and 38% (5). In the study by Avery et al. (5), depression patients and anxiety disorder patients were evaluated. DST was found to be positive in 13% of the depression patients, whereas it was 38% in GAD. In conclusion of the study, the specificity of DST's relation with anxiety disorders, especially with GAD, has been emphasized (5).

In this study, the low dexamethasone suppression of cortisol in GAD may be explained in several ways: Dexamethasone suppression of cortisol is calculated by subtracting the post-DST cortisol level from the baseline cortisol level; baseline cortisol levels in GAD patients are already low, so no matter how much the cortisol is suppressed by dexamethasone, the difference in cortisol level will be low. Interestingly, although baseline cortisol levels were low in the GAD group, post-DST cortisol level was not lower than the other groups. This can be interpreted as nonsuppression. In this study, potential factors that may prevent cortisol suppression with dexamethasone such as benzodiazepine use, associating depression, and weight loss were excluded. Since the patients were hospitalized during the study period, drug incompatibility was out of the question.

In this study, even though the baseline cortisol levels in GAD patients were low, the difference in cortisol with dexamethasone showed that the endogenous cortisol was not sufficiently suppressed by dexamethasone. The lack of suppression of cortisol with dexamethasone is also evident in depression (44). The cause of nonsuppression in depression is considered to be the hypersecretion of

CRH (45). HPA axis hyperactivity in depressed patients has been reported to be associated with a decrease in postsynaptic CRH receptor sensitivity and hypophyseal and adrenal hypertrophy developed secondary to presynaptic CRH hypersecretion (46). It is suggested that there might be alterations in brain corticosteroid receptors due to hyperactive HPA, which may lead to glucocorticoid resistance, as a result of which, glucocorticoid hormones cannot have the necessary feedback effect on different levels of the HPA axis, leading to persistent HPA axis hyperactivation (44). Since hypothalamic glucocorticoid receptors' (GR) sensitivity to cortisone decreases, no negative feedback effect arises, this results in increased hypothalamic CRH release (47). The reduced number of GR in mononuclear cells in depressed patients and its restitution by treatment also supports the presence of GR resistance (48). A nonsuppression may occur in GAD due to similar mechanisms. Or, if our trauma hypothesis is true, the initial CRH increase may have resulted in excessive initial HPA axis activity causing a persistent downregulation in the GR—which is responsible for the negative feedback in the brain. Permanent CRH increase may not increase cortisol because it may have caused a downregulation in CRH receptors. So and so, increased levels of cerebrospinal fluid CRH have been reported in PTSD (49). On the other hand, as in the depression, if the GR down-regulation is a feature of the GAD (44) nonsuppression may be a result of this condition as well. So whether it's a characteristic or a result of the trauma, there seems to be a downregulation in the GR. Nonsuppression of cortisol with dexamethasone may be due to this fact.

In this study, baseline DHEA-S levels in PD and GAD patients were not different from controls. In PD cases, there are reports indicating normal (17) as well as high baseline DHEA (18). However, studies that found normal DHEA values were done in women, and those that found high were made in men. In a study involving 8 male patients with GAD and 8 healthy controls, DHEA-S levels were found to be indifferent from controls (12). The findings of our

study are similar to the studies (12,17) in which DHEA-S level was found similar to controls in PD and GAD. The causes of different values of DHEA-S may be as follows: DHEA-S may have inter-individual variation and may decrease by age in the same individual (50). The differences in blood drawing time may also be another reason for the change in DHEA-S levels (51).

In this study, post-DST DHEA-S levels in PD and GAD were not different from controls. To the best of our knowledge, there are no studies in the literature evaluating the DHEA-S response to DST in PD and GAD patients. Although it is not known exactly how DHEA secretion is regulated, it is suggested that ACTH is partly responsible for the release of DHEA and that dexamethasone may suppress DHEA levels by a mechanism independent from ACTH (52). There may be DHEA secretories independent from ACTH, from unknown locations. A substance called adrenal androgen-releasing hormone which is released from the pituitary has been proposed (53).

Statistically more significant results might be achieved if the number of subjects included in the study were more. The questioning of the trauma history in patients may have facilitated the interpretation of the difference in cortisol values. A more precise assessment would have been possible by measuring free cortisol levels instead of total cortisol. In recent years, it has been suggested that in the assessment of HPA axis, assessment of ACTH and cortisol response to CRH along with DST, in other words, concurrent execution of the CRH stimulation test and DST, is more sensitive than detecting HPA axis abnormalities (11,54). Therefore, in order to assess whether there is an abnormality in the HPA axis or not, implementation of these tests in addition to the DST might have allowed us to obtain more valid results.

In GAD patients, lower baseline cortisol levels may indicate HPA axis hypoactivity with CRH receptor downregulation as a result of CRH hyperactivity, while less suppression of cortisol with DST suggests a downregulation of GR.

Contribution Categories	Name of Author
Development of study idea	Y.H., E.E.
Methodological design of the study	Y.H., E.E.
Data acquisition and process	Y.H., E.E.
Data analysis and interpretation	Y.H., E.E.
Literature review	Y.H., E.E.
Manuscript writing	Y.H., E.E.
Manuscript review and revision	Y.H., E.E.

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