Change in Blood Cortisol Levels During 6-Month Methylphenidate Treatment in Children with ADHD

Dilek Altun Varmiş, MD,[1] Ayşegül Yolga Tahiroğlu, MD,[2] Gamze Yapça Kaypakli, MD,[3] Ayse Avci, MD,[2] Gonca Gül Çelik, MD,[2] Özge Metin, MD,[2] and Selcuk Matyar, MD[4]

[1] *Department of Child and Adolescent Psychiatry, Adana Ekrem Tok Mental Health Hospital, Adana, Turkey*
[2] *Department of Child and Adolescent Psychiatry, Faculty of Medicine, Cukurova University, Adana, Turkey*
[3] *Department of Child and Adolescent Psychiatry, Hatay State Hospital, Hatay, Turkey*
[4] *Department of Biochemistry, Faculty of Medicine, Cukurova University, Adana, Turkey*

ABSTRACT

Background: We sought to investigate the changes in hypothalamic-pituitary-adrenal (HPA) axis after methylphenidate (MPH) treatment, and their association with attention deficit hyperactivity disorder (ADHD) symptomatology.

Methods: We included 86 children with ADHD and 58 healthy controls in this study. Morning cortisol was measured at baseline, 1st, 3rd, 6th month of MPH treatment. K-SADS-PL and Conners' Parent/Teacher Rating Scales (CTRS and CPRS) were collected at each visit.

Results: There was an increase with borderline significance (p=0.054) in cortisol levels measured before and after the 6th month of MPH treatment. However, there was no significant change in control group (p > 0.05). In gender subgroups there was a significant change in boys (p = 0.008), whereas no similar increase was found in girls (p > 0.05). While there was a significant increase in cortisol levels of ADHD combined (ADHD-C) type, no significant change was found in ADHD inattentive (ADHD-I) type. Boys had higher rates of improvement in most of the subscales. While there was a significant decrease in all subscale scores of CTRS and CPRS in ADHD-C group, there was no significant difference in most subscales in ADHD-I group.

Conclusions: Change in plasma cortisol levels may be useful in predicting the long-term treatment response, especially in the male gender and ADHD-C subgroup.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by inattention (IN), hyperactivity (H), impulsivity, and often accompanied by behavioral problems (1, 2). Although the certain etiology of ADHD remains still unknown, one of the hypotheses that plays a role in the pathophysiology of ADHD is a dysfunction of hypothalamic-pituitary-adrenal (HPA) axis (3, 4). Children diagnosed with ADHD have been reported to have abnormalities along with the diurnal rhythm, the response to the dexamethasone suppression test and the cortisol response to stress (3, 5), and they have also been reported to have lower morning cortisol levels than healthy controls (4, 6, 7). Glucocorticoids have regulatory effects on dopaminergic transmission in the brain. Dopaminergic neurons play an important role in ADHD pathophysiology and carry glucocorticoid receptors (GR) which regulates HPA activity. A decrease in GR and mineralocorticoid receptors decreases negative feedback of glucocorticoids and increases HPA activity (8, 9). Glucocorticoids increase the noradrenaline and dopamine levels in the synaptic cleft by binding to GRs on the cell membrane and limiting the monoamine carrier protein activity (10). Therefore, the reduction in cortisol levels may result in increased activity of the monoamine carrier protein, which may cause problems such as IN, H and impulsive behaviors by reducing noradrenaline and dopamine concentrations in the synaptic cleft (11).

Methylphenidate (MPH), the first line treatment of ADHD, increases the neurotransmitter concentrations such as noradrenaline and dopamine in the synaptic cleft

Address for Correspondence: ✉ Gamze Yapca Kaypakli, MD, Specialist in Child and Adolescent Psychiatry, Department of Child and Adolescent Psychiatry, Hatay State Hospital, Hatay, 31001 Turkey gamzeyapca@gmail.com

by reducing the activity of the monoamine carrier protein (12). In light of this, long-term MPH treatment may increase ACTH release and cortisol levels by increasing the noradrenaline and dopamine concentrations in the synaptic cleft, and this increase in cortisol levels may be a sign of response or resistance to treatment. However, there is a very limited number of studies on the association of MPH with HPA axis. Although cortisol levels in patients with ADHD are frequently investigated in cross-sectional studies, there is a small number of studies with conflicting findings that investigated the association of MPH with cortisol levels. It has been stated that acute MPH administration may increase cortisol secretion, but plasma cortisol levels are not affected in long-term applications (13, 14). There have been only two studies which evaluate ADHD symptomatology and cortisol levels of children with ADHD who received MPH treatment (15, 16). In the study of Wang et al. (15), patients treated with MPH for 6 months and untreated healthy controls were compared. Cortisol levels were found to be elevated only in the first month and there was no significant change in cortisol levels within the 6-month period (15). Lee et al. (16) also failed to show any difference between cortisol levels before and after MPH treatment. In the present study, we hypothesized that increase in cortisol levels after MPH treatment should be associated with ADHD symptomatology.

METHODS AND MATERIALS

STUDY SETTING AND SUBJECTS

STUDY POPULATION

In this prospective observational study, we included 86 patients (mean age: 8.9 ± 1.7 years; female; n = 30, 34.9%; male; n = 56, 65.1%) who met the DSM-IV-TR criteria for ADHD and 58 age matched healthy controls (mean age: 10.1 ± 1.3 years; male; n = 26, 44.8%; female; n = 32, 55.2%). Among 100 patients who were initially approached, eight patients, due to risperidone use with conduct disorder comorbidity, five patients due to reluctance to use drugs and one patient with a previous MPH use, were excluded from the study. The treatment center is a university hospital where a large number of ADHD patients are regularly followed. Patients with a pre-diagnosis of ADHD were consulted by the first author, who also evaluated all patients included in the study. ADHD diagnosis was based on DSM-IV and K-SADS-PL which is a reliable semi-structured diagnostic interview. Patients were included if they were newly diagnosed with ADHD and between the ages of 6 and 12. They were excluded if they had treated by cortisol or any psychiatric medication before and any comorbid psychopathology other than oppositional defiant disorder (ODD). As ODD and ADHD very frequently exist together, excluding ODD cases would significantly limit the sample of the study.

The diagnosis of mental retardation, epilepsy, autism spectrum disorder, progressive neurological disease, drug abuse, psychotic disorder, mood disorder, chronic medical disease and having a history of severe head trauma in the last 12 months were other exclusion criteria. Exclusion criteria for control group were the presence of any medical or psychiatric disease, history of cortisol or ADHD treatment and growth or mental retardation. Patients with at least 6 of 9 inattention criteria were accepted as an ADHD-I subgroup, those meeting at least 6 of the hyperactivity criterion as an ADHD-H subgroup, and those meeting 6 criteria from both inattention and hyperactivity as an ADHD-C subgroup. After the approval of the study protocol by the institutional ethics committee, written informed consent was obtained from all patients and their parents. Blood samples were taken to detect the serum cortisol levels of the subjects. The participants were visited again at the 1st, 3rd and 6th month and for the control blood samples.

METHYLPHENIDATE TREATMENT

The participants received a drug formula of OROS-MPH once in the mornings, starting with 0.7-1 mg/kg and one month later titrated the dosage to 1-1.3mg/kg. Patients were advised not to take any additional medication (including painkillers) 24 hours prior to blood collection. Those who experienced mild side effects were advised to continue taking the medication. Those who did not want to continue the medication because of intense anorexia, insomnia or lack of response left the study voluntarily. The osmotic-release oral system (OROS) provides a mixture of immediate and delayed release. Within 1 hour, plasma MPH concentrations reach an initial plateau, followed by a gradual increase over the next 5 to 9 hours. Instead of IR (immediate release), we used OROS MPH in order to provide a single daily dose.

ASSESSMENT INSTRUMENTS AND PROCEDURES

The Sociodemographic Information Form

The semi-structured socio-demographic form was used to determine the past medical history and socio-demographic characteristics of the children and their parents.

Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL)

K-SADS-PL is a reliable semi-structured diagnostic interview for the assessment of a wide range of psychiatric disorders according to DSM-III and DSM-IV-TR criteria (17). In the screening section, the basic symptoms of psychopathologies seen in childhood and adolescence are questioned, and each symptom is evaluated between 0-3 points with its own grading method. A detailed evaluation is assessed in the cases with a score of 3 from any of the symptoms. Cronbach's alpha value of K-SADS-PL is 0.94 (18). Turkish validity and reliability of K-SADS-PL was conducted by Gokler (18).

The Conners' Parent Rating Scale-Revised: Long Form (CPRS-R/L)

The CPRS-R/L comprises 80 items that are subdivided into 14 subscales and assesses both internalizing and externalizing problems in children between the ages of 3 and 17. The scale is used to evaluate the parents' observations on a child's behavior over a 1-month time interval. These subscales are oppositional, cognitive problems/inattention, hyperactivity, anxious-shy, perfectionism, social problems, psychosomatics, ADHD-index, Conners' global index-restless/impulsivity (CGI-RI), Conners' global index-emotional lability (CGI-EL), Conners' global index total (CGI-Total), DSM-IV-inattention, DSM-IV-hyperactivity/impulsivity, and DSM-IV-total (19). The reliability and validity study of the Turkish version was conducted by Kaner et al. (20).

The Conners' Teachers Rating Scale-Revised: Long Form (CPRS-R/L)

The CTRS-R/L comprises 59 items subdivided into the subscales that are oppositional, cognitive problems/inattention, hyperactivity, anxious-shy, perfectionism, social problems, ADHD-index, Conners' global index-restless/impulsivity (CGI-RI), Conners' global index-emotional lability (CGI-EL), Conners' global index total (CGI-Total), DSM-IV-inattention, DSM-IV-hyperactivity/impulsivity, and DSM-IV-total (21). Each item offers four options and is scored between 0 and 3 (Never / rarely: 0 point; Sometimes: 1 point; Most of the time / Often: 2 points; Very Often: 3 points). The severity of symptoms increases with higher scores obtained from the scale. The reliability and validity study of the Turkish version was conducted by Kaner et al. (22).

Plasma Cortisol Levels: Blood samples for cortisol level measurements were collected from ADHD and control groups at the beginning of the study and at the 1st, 3rd and 6th month. In order to provide a reliable way to measure cortisol levels, all participants were advised to fast the night before and to avoid excessive exercise over the 24-hour period before testing. Blood samples were taken before OROS-MPH taken in the morning. This procedure was performed each morning between 9:00 am and 10:00 am and blood samples were sent to the laboratory within 30 minutes. The serum samples were centrifugated at 4,000 rpm for 5 minutes and were evaluated with the chemi luminescence system in Beckman Coulter DXI 800 Access immunoassay auto analyzer. Reference range is determined as 6.7-22.60 µg/ dL.

STATISTICAL ANALYSIS

Statistical analyses were conducted using SPSS, version 16.0 (SPSS Inc. Chicago, Illinois). Data are expressed as mean ± SD for continuous variables and percentage for categorical variables. The Shapiro-Wilk test was used to test normality and a p value >0.05 was defined as normally distributed data. Continuous variables that showed normal distribution were compared using the Student t test and ANOVA, whereas the Mann-Whitney U test and Kruskal-Wallis test were used for non-normally distributed samples. Associations of the categorical variables between groups were tested using the chi-square test. The Wilcoxon test was used to analyze the dependent variables such as the mean scores of psychometric scales obtained by applying the same measurement tool more than once. Pearson's and Spearman's correlations were used to examine the relationship between continuous variables. Statistical significance was defined as a p value <0.05 for all comparisons.

RESULTS

A total of 86 ADHD patients (mean age: 8.9 ± 1.7 years; female; n = 30, 34.9%; male; n = 56, 65.1%) and 58 healthy controls (mean age: 10.1 ± 1.3 years; male; n = 26, 44.8%; female; n = 32, 55.2%) were enrolled to this study. There was no significant difference between the mean age of male and female subjects in the patient and control groups (p> 0.05).

COURSE OF THE BLOOD CORTISOL LEVELS AT 6-MONTH FOLLOW-UP

Blood samples for cortisol level measurements were collected from ADHD and control groups at the beginning of the study and at the 1st, 3rd and 6th month. Of the 86 patients in the ADHD group; blood samples

and psychometric measurements were conducted in 74 (86.0%), 62 (72.1%), and 50 (58.1%) patients for the second, third and fourth visit respectively. In the second visit, 8 patients due to lack of appetite and 4 patients due to sleep deprivation were dropped from the study. In the third visit, 5 patients due to lack of appetite, 5 patients due to lack of response and 2 patients due to sleep deprivation were dropped from the study. In the fourth visit, 9 patients due to lack of response, 2 patients due to lack of appetite and 1 patient due to sleep deprivation were dropped from the study. In the control group, blood samples and psychometric measurements were conducted in 58 (100.0%), 55 (94.8%), and 54 (93.1%) patients for the second, third and fourth visit respectively. The comparison of mean plasma cortisol levels before treatment and at the 1st, 3th and 6th month of treatment at ADHD-control groups and subgroups according to sex, ADHD subtype and comorbidity is shown in Table 1. Figure 1A and B show the course of the blood cortisol levels over time in the patients with ADHD and the healthy controls.

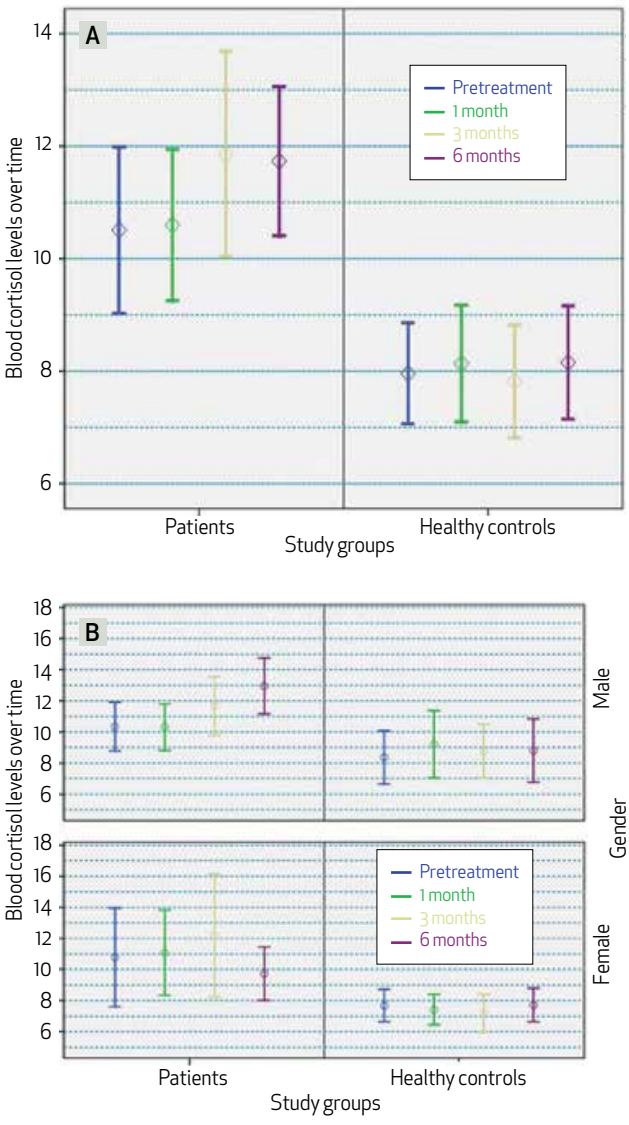
Although there was an increase with borderline significance (-1.2±5.2, p=0.054) in the levels of cortisol measured before and after the treatment of ADHD patients at the 6th month of MPH treatment, there was no significant change in the control group (p > 0.05). When

Table 1. *Course of the morning blood cortisol levels*

	Before Treatment	1st month	3rd month	6th month
ADHD	10.4±5.2	10.3±4.7	11.3±6.1	11.7±4.6
Healthy controls	8.2±3.2	8.3±3.9	8.0±3.6	8.2±3.5
P	0.007	0.006	0.001	0.001
Gender				
Boys	10.2±4.6	9.9±4.5	10.1±4.6	11.2±5.0
Girls	8.6±4.5	8.8±4.3	9.3±6.0	8.5±3.3
P	**0.014**	0.108	0.116	0.013
ADHD				
Boys	10.8±5.0	10.3±4.4	10.7±3.8	12.9±4.8
Girls	9.7±5.6	10.2±5.2	12.3±7.2	9.7±3.5
P^1	0.155	0.591	0.741	**0.021**
Healthy controls				
Boys	8.5±3.6	9.2±4.7	9.1±5.0	8.7±4.3
Girls	7.6±2.8	7.6±2.9	7.3±3.3	7.9±2.9
P^2	0.171	0.412	0.061	0.585
P^3	0.160	0.182	0.229	**0.002**
P^4	0.087	**0.047**	**0.005**	0.073
ADHD population				
ADHD-C	10.5±5.6	10.0±4.7	10.8±5.5	12.0±4.7
ADHD-I	10.3±2.9	11.2±4.6	13.1±7.8	10.4±4.1
P	0.442	0.245	0.283	0.410
ODD Comorbidity				
With	10.8±6.1	10.3±4.8	10.9±5.7	11.8±5.0
Without	10.2±3.6	10.4±4.7	12.0±6.7	11.5±4.3
P	0.729	0.768	0.640	0.984

Man-Whitney U Test, **p1:** Comparison of genders in children with ADHD; **p2:** Comparison of genders in healthy controls; **p3;** Comparison of boys in ADHD and healthy controls; **p4:** Comparison of girls in ADHD and healthy controls. **ODD:** Oppositional defiant disorder

Figure 1. **A.** *The course of the blood cortisol levels over time in the patients with ADHD and the healthy controls*
B. *The course of the blood cortisol levels over time according to gender groups*



similar analyses were repeated in gender subgroups there was a significant change in the boys at the 6th month of treatment compared to baseline (- 2.6 ± 5.2, p = 0.008). On the other hand, no similar increase was found in the girls (1.0 ± 4.4, p = 0.546). Differences between cortisol levels measured before and after the 6th month of treatment in the ADHD and control groups and in the gender subgroups are shown in Table 2. Among the sub-groups of ADHD subtypes, while there was a significant increase in cortisol levels of ADHD-C type at the 6th month of treatment, no significant change was found in ADHD-I type. There was no difference between the cortisol levels of patients with or without comorbid ODD.

COURSE OF THE PSYCHOMETRIC SCALES OF ADHD SYMPTOMS AT 6-MONTH FOLLOW-UP

According to CPRS and CTRS, boys had higher rates of improvement than girls in most of the subscales. The differences of the mean values of psychometric scales between the first and last visits by gender are given in Table 3. Among the patients with ADHD-C, there was a significant decrease in all subscale scores of CTRS, except for perfectionism, and all of the CPRS sub-scores at the 6th month of treatment. Similar analyses in the ADHD-I group showed no significant difference in most of the subscales of all three scales. The details of these analyses and the comparison of the mean scores of the psychometric scales applied in the first evaluation according to ADHD subgroups are given in Table 4.

Table 2. *Comparison of blood cortisol levels within the course of the treatment*

	1-2 Vizit	p	1-3 Vizit	p	1-4 Vizit	p
All study population	0.1±4.4	1.000	-0.4±4.1	0.477	-0.6±4.3	**0.051**
ADHD	0.1±5.4	0.769	-0.9±4.6	0.364	-1.2±5.2	**0.054**
Healthy controls	-0.0±2.7	0.677	0.0±3.3	0.966	-0.1±3.2	0.501
ADHD						
Boys	0.4±6.0	0.368	-0.5±4.1	0.686	-2.6±5.2	**0.008**
Girls	-0.2±4.2	0.409	-1.6±5.4	0.372	1.0±4.4	0.546
Healthy controls						
Boys	-0.3±3.4	0.568	-0.5±3.7	0.260	-0.4±3.2	0.592
Girls	0.2±1.9	0.883	0.5±3.0	0.456	-0.0±3.2	0.659
ADHD subtype						
ADHD-C	0.4±5.7	0.556	-0.4±3.9	0.594	-1.6±5.1	**0.013**
ADHD-I	-0.7±4.1	0.609	-2.5±6.6	0.422	0.5±5.3	0.646
ODD Comorbidity						
With	0.1±6.3	0.991	-0.7±4.3	0.432	-1.4±5.5	0.099
Without	0.3±3.8	0.548	-1.1±5.2	0.819	-0.6±4.8	0.434

ODD: Oppositional defiant disorder

Correlation analysis of the relationship between blood cortisol levels and psychometric scales of ADHD (Table 5)

In the evaluation of the correlation between the cortisol change and the change of psychometric scores in the bivariate analysis we found a negative and significant correlation with only the CPRS-EL (emotional lability) score. Scatter plot analysis of the relationship between the change in the Conners' family emotional lability score and cortisol change is shown in Figure 2.

DISCUSSION

To the best of our knowledge, this is the first study that demonstrates the change in plasma cortisol levels after MPH treatment and the correlation of this change with ADHD symptomatology. There are limited data regarding the association of MPH with the HPA axis. These studies mostly used saliva samples to evaluate cortisol levels. Unlike previous studies, cortisol levels were measured from blood samples in our study.

Basal plasma cortisol levels, measured in the mornings, were reported to be lower in the ADHD group than healthy individuals (4, 6). In our study, basal cortisol levels were significantly higher in the ADHD group compared to the healthy children. This finding was in contrast to findings of previous studies. One possible explanation could be the circadian rhythm of the cortisol secretion. Although the time intervals for sampling in our study were similar to the previous studies, the differences of study population such as gender and waking time may contribute to this issue. Another explanation may be the sample technique. Since previous studies used salivary cortisol measurements, the use of blood cortisol levels may be responsible for higher cortisol levels in the ADHD population.

The certain effects of MPH on the HPA axis are not yet known. Although cortisol levels in ADHD have been discussed in many studies in the literature, to the best of our knowledge there is only one prospective study comparing and examining the relationship between cortisol levels and MPH treatment response in ADHD patients and healthy children. Wang et al. (15) investigated salivary cortisol levels measured before, and at 1st, 3rd and 6th month of treatment compared with healthy controls (15), and found a significant increase in patients with ADHD after 1-month MPH treatment compared to the healthy population. However, cortisol levels decreased

Table 3. *Psychometric scale scores by gender in ADHD, differences between first and last measurement scores*

	First measurement scores			Comparison of the first and last measurement scores			
	Boys	Girls	p[1]	Boys	p[2]	Girls	p[2]
CPRS							
Oppositional	11.6±7.4	11.2±6.2	0.899	5.1±6.9	0.0001	4.5±7.6	0.024
Inattention	19.9±8.5	19.5±7.4	0.730	9.6±8.6	**0.0001**	10.6±7.4	**0.0001**
Hyperactivity	10.4±6.0	9.0±5.5	0.276	5.8±5.4	**0.0001**	4.6±5.7	**0.003**
Anxious-shy	7.0±5.1	8.2±4.7	0.224	3.6±4.0	0.0001	3.4±5.0	**0.011**
Perfectionism	6.2±4.1	5.9±2.7	0.877	2.1±4.2	**0.008**	1.9±3.4	**0.016**
Social problems	3.4±3.0	3.9±3.1	0.398	1.4±3.1	**0.004**	1.9±2.4	**0.005**
Psychosomatics	3.5±4.1	5.9±3.4	**0.001**	1.7±4.7	0.063	3.5±4.4	**0.004**
ADHD-index	21.3±8.5	20.4±7.7	0.508	11.3±9.4	**0.0001**	11.5±7.6	**0.0001**
CGI-RI	10.2±5.3	9.2±5.0	0.347	5.6±5.1	**0.0001**	4.8±4.4	**0.002**
CGI-EL	3.3±2.6	3.9±2.3	0.273	1.4±2.3	**0.002**	1.5±2.3	**0.014**
CGI-Total	13.5±7.4	13.1±6.9	0.842	7.0±6.8	**0.0001**	6.4±6.1	0.001
DSM-IV-inattention	14.9±6.6	14.1±5.9	0.396	7.1±7.1	**0.0001**	7.6±6.4	**0.001**
DSM-IV-hyperactivity	12.9±7.1	10.9±7.5	0.212	7.6±6.9	**0.0001**	5.9±7.0	**0.002**
DSM-IV-total	27.8±12.2	25.1±12.2	0.269	14.7±12.6	**0.0001**	13.6±11.7	**0.0001**
CTRS							
Oppositional	5.0±4.8	1.7±1.8	**0.001**	2.2±3.9	**0.005**	1.0±2.3	0.087
Inattention	11.5±6.2	8.8±6.0	**0.037**	5.9±5.7	**0.0001**	5.5±5.5	**0.0001**
Hyperactivity	9.6±5.3	4.8±4.9	**0.0001**	6.4±4.7	**0.0001**	1.9±4.3	0.097
Anxious-shy	6.3±3.7	7.0±3.5	0.259	1.7±4.1	0.094	4.5±3.5	**0.0001**
Perfectionism	3.3±3.0	5.4±3.5	**0.006**	-0.6±3.8	0.468	2.4±2.5	**0.001**
Social problems	6.5±4.0	3.7±3.7	**0.002**	4.8±3.7	0.0001	3.2±4.0	**0.005**
CGI-RI	8.7±4.0	5.4±4.1	**0.001**	5.7±3.6	**0.0001**	2.9±3.2	**0.002**
CGI-EL	5.4±3.2	3.6±2.3	**0.012**	3.0±3.4	**0.0001**	1.5±2.0	**0.007**
CGI-Total	14.1±6.7	9.0±5.9	**0.001**	8.7±6.5	**0.0001**	4.5±4.3	**0.001**
DSM-IV-inattention	17.3±6.2	12.2±7.0	**0.002**	9.5±5.8	**0.0001**	7.1±7.0	**0.001**
DSM-IV-hyperactivity	13.5±8.0	6.0±6.6	**0.0001**	9.5±7.6	**0.0001**	3.1±5.5	0.057
DSM-IV-total	29.2±11.3	17.5±11.1	**0.0001**	18.1±10.6	**0.0001**	10.3±8.8	**0.001**

p[1]: Man-Whitney U Test, Comparison of the mean scales applied at the first evaluation of male and female subjects diagnosed with ADHD; **p[2]:** Wilcoxon Test, Analysis of the differences between the scale scores applied at the 1st and 4th evaluation of boys and girls diagnosed with ADHD, **CPRS:** Conners Parents Rating Scale, **CTRS:** Conners Teacher Rating Scale; **CGI-RI:** Conners' global index-restless/impulsivity; **CGI-EL:** Conners' global index-emotional lability; **CGI-Total:** Conners' global index total

after the 6-month treatment and reached the level of healthy controls. The authors explained it as tolerance to MPH treatment. In another study, Wang et al. (23) have found no significant difference in salivary cortisol levels in patients diagnosed with ADHD which were evaluated before and after 6 months of MPH treatment (23). Our study is different from previous studies in terms of using plasma cortisol levels as previous studies mostly used salivary cortisol levels. Furthermore, our study is first to demonstrate the change in plasma cortisol levels after MPH treatment. The previous two studies of Wang et al. (15, 23), the study of Lee et al. (16) and the study of Isaksson et al. (7) failed to demonstrate the change in plasma cortisol levels after MPH treatment. Our study is also the first to clearly verify the negative correlation of ADHD symptoms with cortisol plasma levels.

GENDER DIFFERENCES

In a single prospective study which investigates the effects on cortisol levels during MPH treatment, the number of girls is insufficient for statistics. Our study is important in this respect. We found that although there was a significant increase in cortisol levels of the boys with ADHD at the end of the 6-month treatment, no significant relationship was found in girls with ADHD (Figure 1B). It is shown to have a mutual relationship

Table 4. *Psychometric scale scores according to subtypes, differences between first and last measurement scores*

	First measurement scores			Comparison of the first and last measurement scores			
	ADHD-C	ADHD-I	p[1]	ADHD-C	p[2]	ADHD-I	p[2]
CPRS							
Oppositional	12.4±7.0	7.7±5.5	0.006	5.7±6.8	0.0001	1.9±7.8	0.414
Inattention	20.6±8.4	16.4±6.0	**0.019**	10.7±8.0	**0.0001**	7.1±8.1	0.028
Hyperactivity	11.4±5.4	4.4±3.6	**0.0001**	6.4±5.5	**0.0001**	1.4±3.7	0.360
Anxious-shy	7.5±4.9	7.1±5.3	0.562	3.8±4.2	**0.0001**	2.6±4.9	0.152
Perfectionism	6.6±3.8	4.3±2.6	**0.036**	2.3±4.2	**0.002**	1.1±2.1	0.134
Social problems	3.7±3.0	2.8±2.8	0.197	1.5±3.0	**0.001**	2.1±2.1	**0.011**
Psychosomatics	4.6±4.2	3.6±3.2	0.524	2.7±4.9	**0.002**	1.3±3.1	0.205
ADHD-index	22.4±8.3	15.6±5.1	0.0001	12.3±8.9	**0.0001**	7.8±7.1	**0.012**
CGI-RI	10.9±5.2	5.8±2.4	**0.0001**	5.9±5.0	**0.0001**	2.9±3.3	**0.028**
CGI-EL	3.9±2.5	1.8±1.7	**0.002**	1.6±2.5	**0.0001**	0.8±1.6	0.155
CGI-Total	14.9±7.2	7.7±3.7	**0.0001**	7.6±6.7	**0.0001**	3.7±4.1	**0.012**
DSM-IV-inattention	15.3±6.5	12.0±4.9	**0.036**	7.8±7.0	**0.0001**	5.2±5.6	**0.016**
DSM-IV-hyperactivity	14.3±6.3	4.2±4.4	**0.0001**	8.5±6.8	**0.0001**	0.7±3.1	0.512
DSM-IV-total	29.7±11.8	16.2±7.1	**0.0001**	16.4±12.4	**0.0001**	5.9±6.1	**0.007**
CTRS							
Oppositional	4.4±4.6	1.7±1.6	**0.040**	2.0±3.7	**0.002**	0.8±1.9	0.228
Inattention	11.0±6.2	8.8±6.2	0.131	5.5±5.5	**0.0001**	6.9±6.2	**0.011**
Hyperactivity	9.2±5.4	2.8±3.1	**0.0001**	5.8±4.9	**0.0001**	0.4±2.1	0.552
Anxious-shy	6.0±3.4	8.7±3.6	**0.005**	1.9±3.7	**0.005**	6.2±4.2	0.007
Perfectionism	3.9±3.4	4.6±3.3	0.285	0.1±3.4	0.581	2.1±4.4	0.141
Social problems	5.9±4.1	4.2±3.8	0.141	4.3±3.9	**0.0001**	3.9±3.9	**0.025**
CGI-RI	8.5±4.2	4.0±2.9	**0.0001**	5.1±3.8	**0.0001**	2.8±2.7	**0.012**
CGI-EL	5.1±3.1	3.3±2.1	**0.022**	2.7±3.0	**0.0001**	1.6±2.6	0.134
CGI-Total	13.6±6.8	7.3±4.4	**0.0001**	7.8±6.2	**0.0001**	4.4±4.7	**0.017**
DSM-IV-inattention	16.2±6.6	13.0±7.7	0.103	8.4±5.6	**0.0001**	9.4±8.8	**0.011**
DSM-IV-hyperactivity	12.9±7.5	3.2±4.7	**0.0001**	8.7±7.3	**0.0001**	0.5±3.5	0.714
DSM-IV-total	27.6±12.0	15.7±9.9	0.0001	16.5±10.6	**0.0001**	9.8±9.2	**0.022**

p[1]: Man-Whitney UTest; Comparison of the mean scales applied at the first evaluation of subtypes of ADHD; **p[2]:** Wilcoxon Test, Analysis of the differences between the scale scores applied at the 1st and 4th evaluation of subtypes of ADHD; **CPRS:** Conners Parents Rating Scale, **CTRS:** Conners Teacher Rating Scale; **CGI-RI:** Conners' global index-restless/impulsivity; **CGI-EL:** Conners' global index-emotional lability; **CGI-Total:** Conners' global index total

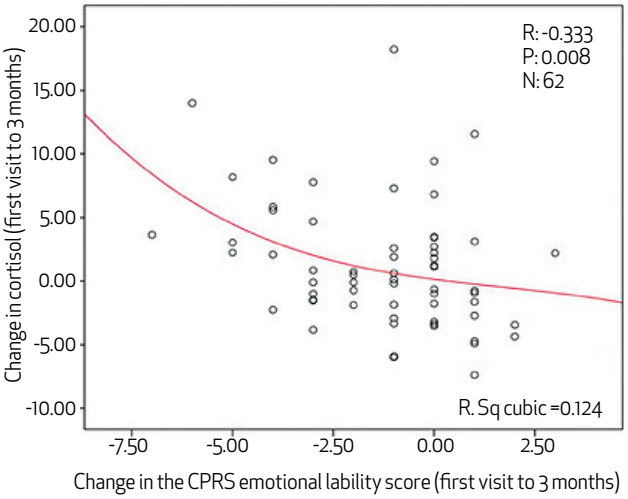
on HPA axis and hypothalamus-pituitary-testis axis functions (24, 25). It was reported in a study on rats that pre-puberty MPH treatment may lead to an increase in corticosterone and testosterone levels in the long term with the enriched environment (26). The increase of cortisol levels especially seen in boys after treatment can be explained by this linkage. It is suggested that differences related to cognitive function between genders can be explained by exposure to different levels of reproductive hormones during the fetal period. Furthermore, there is some evidence that the effects of these hormones on cognitive functions can be long lasting. Variable levels of attention and concentration at different periods of the menstrual cycle are a good example to this issue (27, 28). The gender differences obtained from our study were noteworthy due to clinical differences between the genders according to the CTRS during the first evaluation and the explicit improvement of both the CPRS and CTRS (Table 3). Additionally, the differences in response to MPH treatment compared to gender and the correlation of cortisol levels with the response of MPH treatment were also striking. The relationship between cortisol and other steroid hormones, which are notable as a factor with different roles from ADHD etiology to drug responses, should be investigated in future studies.

It is also known that cortisol, stress and reproductive hormones play a major role in each other's levels

Table 5. *Bivariate correlation analysis of the relationship between change in blood cortisol levels from first to last measurement and change in psychometric scales of ADHD*

	Change in blood cortisol	
	R	P
CPRS		
ADHD-index	-0.132	0.305
CGI-RI	-0.090	0.408
CGI-EL	-0.333	**0.008**
CGI-Total	-0.200	0.119
DSM-IV-inattention	-0.142	0.270
DSM-IV-hyperactivity	-0.162	0.207
DSM-IV-total	-0.170	0.187
CTRS		
CGI-RI	0.110	0.395
CGI-EL	-0.041	0.752
CGI-Total	0.052	0.691
DSM-IV-inattention	0.066	0.613
DSM-IV-hyperactivity	-0.055	0.672
DSM-IV-total	-0.029	0.822

Figure 2. *Scatter plot analysis of the relationship between the change in the Conners' family emotional lability score and cortisol change*



and activities as well (29, 30). Lack of measurements of reproductive hormones in this study is an important limitation. Considering the differences between the genders, there is a need for future pre-clinical and clinical studies evaluating the association of cortisol and reproductive hormones that can provide information to fill important gaps in our knowledge of ADHD.

ADHD SUBTYPES

There are significant differences between ADHD-IN and ADHD-C subtypes, including clinical features, long-term course and treatment responses (31-33). Detailed information from future studies regarding ADHD subtypes may be beneficial in terms of the identification of the clinical subgroups with various risk factors, planning of case-specific treatments, better prediction of the efficacy and side effects, and the development of new drug therapies with more specific goals (34-36). In our study, plasma cortisol levels significantly higher in ADHD-C patients after the 6-month MPH treatment and clinical improvement was also noteworthy. However, there was no significant difference in ADHD-IN patients after treatment.

Emotional lability is an important condition in which individuals with ADHD experience difficulties (37). In the study of Popma et al. (30), there was a significant interaction between cortisol and testosterone in relation to overt aggression, with a significant positive relationship between testosterone and overt aggression in subjects with low cortisol levels but not in subjects with high cortisol levels (30). Although there are no studies in the literature investigating the relationship between emotional lability and cortisol in children with ADHD, it was hypothesized that HPA axis dysfunction due to negative psychosocial stimuli may be a marker for emotional dysregulation (38, 39). In a previous study with adults with drug-naïve ADHD, Ramos-Quiroga et al. (39) found highest cortisol levels were associated with the greatest difficulties in regulating emotion in inattentive patients, whereas, in the combined subtype, although not statistically significant, the correlations between emotional lability and cortisol were negative (39). In our study, clinical improvement seems to be related to the increase in cortisol levels after treatment. The correlation between clinical improvement and cortisol change was found to be most significant in emotional lability (Figure 2).

THE ASSOCIATION OF ODD COMORBIDITY WITH THE COURSE OF PLASMA CORTISOL

In many studies, low cortisol levels were found to be associated with comorbid ODD (40-42). In a previous study, it was stated that ODD comorbidity based on clinical assessment could predict the level of cortisol. However, the authors did not find any meaningful results when they analyzed the results of psychometric scales from the evaluations of parents and teachers (43). In this study, though, there was no significant difference between patients with and without ODD.

CONCLUSION

Although there are many studies investigating the relationship between cortisol and ADHD, there are very few that provides follow-up data including control subjects or addressing the response of MPH treatment. The results of our study suggest that cortisol measurements may be useful in predicting long-term treatment response, especially in the male gender and ADHD-C subgroup. In our study, increased cortisol levels were associated with clinical improvement after 6 months of treatment. On the other hand, the question of the possible effects of cortisol on the efficacy of MPH treatment remains to be answered.

Future studies are needed to understand how cortisol plays a role as a mediator on the therapeutic effects of MPH. Furthermore, studies with large samples with well-defined patients in terms of ADHD severity and subtypes may provide valuable information to demonstrate the validity of basal cortisol levels in clinical practice for diagnosis and prediction of MPH response.

LIMITATIONS

Since the release of cortisol during the day varies, the waking time of the participants is important, and in this study the waking time which can affect the cortisol levels was not precisely identified. We did not evaluate some variables that could affect plasma cortisol such as BMI and body weight. Furthermore, although the number of participants is statistically sufficient for both sexes, studies with larger samples are needed.

CLINICAL SIGNIFICANCE

The current study identifies the potential for use of blood cortisol levels and their change with MPH treatment to predict the response to therapy in patients with ADHD. In this study, we suggest two major clinical implications: Firstly, cortisol levels were found to be increased in patients with ADHD. However, this increase is mainly based on male patients and patients with ADHD-C subtype. Secondly, change in cortisol levels were found to be correlated with significant improvement in emotional lability.

The study was approved by the institutional ethics committee of Cukurova University (Adana, 2014, reference: 26/20)
Written informed consent was obtained from all patients and their parents.
The authors have nothing to disclose.
The authors report no conflict of interest.

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