D J Med Sci 2024;10(3):113-122
doi: 10.5606/fng.btd.2024.158

Original Article

Comparison of HPA axis responses to psychological stress in recently abstinent male patients with alcohol dependence, their unaffected first-degree relatives, and healthy controls

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ABSTRACT

Objectives: In this study we aim to compare the adrenocortical responses to psychological stress of recently abstinent alcohol-dependent patients, their unaffected first-degree relatives, and control participants.

Patients and methods: Between January 2010 and June 2010, a total of 94 participants, alcohol-dependent group (32 males; mean age: 45.9±8.6 years; range, 18 to 65 years), first-degree relatives group (32 males; mean age: 41.7±9.4 years; range, 18 to 65 years), and control group (30 males; mean age: 41.0±8.5 years; range, 18 to 65 years) were included in the data analyses. All patients met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for alcohol dependence and had been abstinent for >3 weeks. Controls and first-degree relatives had no reported history of DSM-IV Axis I disorders, including any substance use disorder. Participants reported to the general clinical research center study room at 9:00 to undergo a psychological stress test consisting of 30 min of public speaking followed by 15 min of mental arithmetic. Two baseline (–30 min, immediately before the beginning of the test) and five post-stress (15, 30, 45, 60, 90 min) blood samples were drawn.

Results: At baseline, cortisol and adrenocorticotropic hormone (ACTH) levels in the alcohol-dependent group were significantly different than those in both the relatives and the healthy controls. Post-stressor cortisol and ACTH values differed between the alcohol-dependent patients and both of their relatives the controls at timepoints +15, +30, +45, +60, and +90. In addition, we found psychological stress-induced ACTH/cortisol rise in the alcoholics, and significant psychological stress-induced ACTH-only rise post-stress (15, 30, 60, 90 min) in their first-degree relatives, as compared to controls.

Conclusion: In agreement with prior studies, we found a deficient activation of the HPA axis in recently abstinent alcoholics than in their first-degree relatives and controls. These findings suggest that HPA hypo-responsiveness may be an important characteristic of individuals prone to alcohol dependence and suggest that the disruption in first-degree relatives occurs at the pituitary level.

Keywords: Abstinent, alcohol dependence, HPA axis, psychological stress.

Alcohol dependence is a disorder that is considered to develop as a result of mutual effects of psychosocial (such as childhood experiences, attitudes of parents, social policies, and culture) and biological (biochemical, and genetic) factors, and may cause social and physical degradation.[1]

Early identification of individuals who are at risk of developing alcohol addiction may ensure that early precautions are taken to protect these individuals from developing alcohol addiction. Determination of risk factors for alcohol addiction may be beneficial for clarifying the cause of alcoholism, determining the sub-groups for the disorder, validating biological markers in these sub-groups, and perhaps finding new treatment methods.[2] For these reasons, especially in the last 20 years, numerous studies have been conducted in order to specify biological trait markers for

Received: October 20, 2024
Accepted: December 12, 2024
Published online: January 24, 2025

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Cite this article as:
Koyuncu Kütük E, Han Almis B, Dilbaz N, Akkişi Kumsar N. Comparison of HPA axis responses to psychological stress in recently abstinent male patients with alcohol dependence, their unaffected first-degree relatives, and healthy controls. D J Med Sci 2024;10(3):113-122. doi: 10.5606/fng.btd.2024.158.

alcohol addiction (character determiners or "trait markers").[3-5]

In the past, studies aiming to specify the trait markers of alcohol addiction are promising in showing possible trait markers in alcoholism or in some sub-groups of alcohol-dependent patients.[3,6-8] These include abnormal adenylate cyclase (AC) activity, decreased monoamine oxidase levels, hypothalamic-pituitary-adrenal (HPA) axis disorders, beta-endorphine (ß-endorphine) abnormalities, low P300 stimulated potential amplitude, low D2 dopamine receptor sensitivity, and low sensitivity to alcohol. All of the above-mentioned factors are considered risk factors for alcohol dependence, with the exception of abnormal aldehyde dehydrogenase isoenzyme patterns, which are considered protective factors.

Prior studies conducted during periods of alcohol abstinence have demonstrated a blunting in HPA axis activation response for both pharmacological and psychological stressors.[9] Moreover, it was reported that individuals who had a family history of alcoholism showed a decreased adrenocorticotropic hormone (ACTH) response to corticotrophin-releasing hormone (CRH).[10] Studies suggesting that HPA axis abnormalities exist before the development of alcoholism and that individuals tend towards alcohol abuse disorders due to these abnormalities are available in the literature.[10,11] It is still uncertain whether the abnormality observed in the response to stress in HPA axis activity is a cause or result of alcohol addiction.

Several studies reporting HPA axis response to psychological stress under laboratory conditions are available.[12,13] In two of these studies, a blunted response to psychological stress for alcohol addicts compared to the control group was observed,[9,12] whereas one study showed no differences between the two groups in terms of the adrenal axis response.[13]

In this study, based on the assumption that HPA axis abnormalities may be trait markers, we compared the adrenocortical responses to psychological stressors among alcohol-dependent patients in the abstinence period, their non-alcohol-dependent first-degree relatives, and individuals in the control group. Adrenocorticotropic hormone and cortisol values, which reflect HPA axis activity, were measured at multiple timepoints both at baseline and after exposure to psychological stressors.

It is alleged that some sorts of HPA axis anomalies in alcoholism may be inherited through genes, thus these people are prone to consuming alcohol due to the HPA axis anomalies that they do possess. This study has been planned regarding the hypothesis that the HPA axis response to psychological stress stimulus has been blunted in the patients that possess the alcohol dependence syndrome and their first-degree unaffected relatives compared to the healthy controls regarding age and gender.

The aim of the study is to detect the people prone to developing alcohol dependence in the first place, designate the risk factors engendering the disposition, take precautions to protect the individuals from alcoholism, elucidate the alcoholism etiology, and pave the way for new possible treatment methods.

PATIENTS AND METHODS

Patient selection

This case control study was conducted at Ankara Numune Training and Research Hospital Alcohol and Substance Addiction Treatment Center Clinic (ANTRH-ASATC) between January 2010 and June 2010. A total of 94 participants, alcohol-dependent group (32 males; mean age: 42.9+8.6 years; range, 18 to 65 years), first-degree relatives group (32 males; mean age: 41.7±9.4 years; range, 18 to 65 years), and control group (30 males; mean age: 41.0±8.5 years; range, 18 to 65 years) were included in the data analyses.

The inclusion criteria for the patient group were as follows: a diagnosis of "alcohol dependence syndrome" according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR); a history of alcohol dependence for at least five years; absence of any other physical illness; and sufficient cognitive capacity to understand the tests and instructions provided. The inclusion criteria for the control group were; being a healthy volunteer participant between the ages of 18 and 65, having no psychiatric disorder according to the DSM-IV, having no abuse and/or dependence on alcohol or other substances, having no known physical

illness, having the cognitive capacity to read and comprehend the tests and instructions and being literate, having not used alcohol for at least 10 days. Exclusion criteria for all participants included: having a diagnosis according to the DSM-IV-TR, having misuse/dependence of substances other than alcohol or cigarettes, being diagnosed with a somatic disease (hypertension, diabetes mellitus, rheumatoid arthritis), having HPA axis or thyroid disease, using a medication that affects metabolism, using a chronic medication, having an endocrine disease and having dementia or other organic mental disorders. Medical diseases of the cases participating in the study were evaluated regarding patients' statements and hospital records. Also, women were excluded from the study because of the influence of cyclic estrogen and progesterone on stress hormones in women. Stress in adulthood mediates HPA activity in females through the activation of a sympathetic neural pathway originating in the hypothalamus and releasing norepinephrine (NE) into the ovary, which produces a non-cyclic anovulatory ovary that develops cysts. In the opposite direction, sex differences and sex steroid hormones regulate the HPA axis. Estrogen appears to decrease serotonin receptor 5-HT1A receptor function at presynaptic sites, yet increase 5-HT1A receptor expression at postsynaptic sites. These mechanisms could explain heightened stress HPA axis responses in females compared to males. Patients, relatives, and individuals in the control group were provided with detailed information on the study by the team conducting the study at ANTRH-ASATC. Written consent was obtained from all participants and their socio-demographic characteristics were recorded by socio-demographic and clinical characteristics form. Afterward consent, the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) was administered. The study protocol was approved by the University Research Ankara Numune Training and Research Hospital Ethics Committee (date: 23.12.2009, no. 2). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Screening

The patients' group met DSM-IV criteria for alcohol dependence and also had been abstinent for 3+ weeks. The first-degree relatives and controls had no reported history of DSM-IV Axis I disorders, including any substance use disorder. Participants are summoned to undergo a psychological stress test consisting of 30 min of public speaking followed by 15 min of mental arithmetics to the general clinical research center study room at 9:00 AM. Two baseline (~30 min, before the beginning of the test) and five post-stress (15, 30, 45, 60, 90 min; after the test) blood samples were drawn.

Before starting the study potential participants' Hamilton Depression Rating level was measured and only those scoring <7 were included in the study.

Stress sessions

The stress test consisted of public speaking tasks[14] and mental arithmetic. The three speeches are prepared and presented sequentially within a 30-min period. Before each speech, the participant has 4 min to prepare and then 4 min to give the presentation. The speech is made to a researcher dressed in a white coat who listens attentively and takes notes while a video camera records the speech. The participant was told that other personnel would be watching the recording to evaluate and discuss the fluency of the presentation. The order of the speech topics is randomly selected for each participant.

The mental arithmetic task is started right after the speech without a break except for short explanations and consists of three sections of 5 min. In this serial addition task, a 3-digit number is given to the participant (e.g., 325) and he or she is asked to add 10 to the given number (e.g., 325+10), and then say the new number (e.g., 335) aloud. This continues for 5 min until the participant is asked to stop. If the participant makes an error, they are stopped and the addition begins from the last correct sum.

Data collection tools

Physiologic measures

At the beginning of the stress test; the blood pressure values of patients, relatives, and individuals in the control group were measured. Afterward, a State-Trait Anxiety Inventory (STAI) was given before stress tests. Before test instruction was given, blood samples were taken at –30 and 0 min for basal values, and the STAI

was administered after the stressor was initiated and finished. Blood pressures were measured once at 0 min and 4 mL blood samples were taken in biochemistry and hemogram tubes at 15, 30, 45, 60, and 90 min. Samples were stored at –29°C until tested at a private center.

Self-report assessments

Structured Clinical Interview for DSM-IV Axis I Disorders is a structured clinical interview scale that was developed to establish Axis I diagnoses. Structured interviews were developed to increase the reliability of the diagnosis by standardizing the assessment process and to systematically evaluate symptoms that are not apparent. The SCID-I is a clinical diagnosis instrument structured by First et al.[15] Adaptation and reliability studies for the Turkish population were conducted by Corapcioglu et al.[16]

Hamilton Rating Scale for Depression measures the level and severity change of depression in the patient. It is a scale applied by the interviewer. It was developed by Hamilton in 1960. The Turkish validity and safety study was performed by Akdemir et al.[17]

The State-Trait Anxiety Inventory was developed by Spielberger et al.[18] and consists of two subscales continuous and state-trait, each of which contains 20 questions. It can be applied to individuals aged over 20. The state-trait anxiety scale determines how the individual feels at a specific time and under specific conditions while the continuous anxiety scale determines how the individual feels independent from the situation and conditions he or she is in. The adaptation, validity, and safety study of State-Trait and Continuous Anxiety Inventories was performed by Oner and Le Compte.[19]

Statistical analyses

The socio-demographic data of groups, as well as means and standard deviations of cortisol and ACTH values were calculated. Descriptive statistics were presented as mean ± standard deviation (SD) for the normally distributed variables. Categorical variables were reported as frequencies and percentages.

Between-group differences were analyzed using one-way analysis of variance (ANOVA). The distribution of data was controlled by the Kolmogorov-Smirnov test. The variables for which intergroup differences were found according to the ANOVA were evaluated by the Tukey test as a paired post-hoc test. Differences between ACTH and cortisol levels at each timepoint were analyzed with two-way ANOVA. Tukey's honestly significant difference test was used as a post-hoc test. Chi-square tests were used to examine differences between groups on socio-demographic variables. The a priori statistical significance level was established at $p<0.05$. Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographics

A total of 94 participants were included in the data analyses; 32 subjects out of the study samples were alcohol-dependent, 32 were first-degree relatives of one of the enrolled alcoholics, and 30 were in the control group. The average age of the subjects was 42.93+9.0 in total. The average age of alcohol-dependent participants was 45.94+8.6, the average age of first-degree relatives was 41.69+9.4, the average age of the healthy control group was 41.03+8.5 and there was no difference between groups ($p=0.064$). All participants were male and each had social security insurance. The socio-demographic features are provided in Table 1.

Self-report scales

The Hamilton rating scale for depression was performed on all of the three groups involved in the study and evaluations of state-trait and continuous anxiety inventory demonstrated no anxiety disorder or major depression in the sampling group.

The ACTH and cortisol levels from blood samples of the study groups in the ranges mentioned above (at baseline and after stress) were analyzed and the values are shown in Tables 2 and 3. At baseline, cortisol levels in the alcohol-dependent group were significantly different than those in both the relatives and the healthy controls ($p=0.008$). Post-stressor cortisol values differed between the alcohol-dependent patients and the controls at timepoints +15 ($p<0.001$), +30 ($p<0.001$), +45

Table 1. Demographic properties per group

	Alcohol addict (n=32)			Relative of addict (n=32)			Control group (n=30)			
Variables	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			45.94±8.6			41.69±9.4			41.03±8.5	0.064
Education level										
Primary education	12	37.5		10	31.3		7	23.3		
High school or equivalent	11	34.4		15	46.9		12	40.0		0.421
College or University	9	28.1		7	21.9		11	36.7		
Marital Status										
Married	18	56.3		22	68.8		12	40.0		0.075
Other	14	43.8		10	31.3		18	60.0		
Living Arrangements										
With the family	28	87.5		31	96.9		28	93.3		
Single	3	9.4		1	3.1		6	6.4		0.247
Other	1	3.1		-	-		1	1.1		
Employment Status										
Unemployed	2	6.3		2	6.3		-	-		
Employee	28	87.5		28	87.5		30	100		0.393
Other	2	6.3		2	6.3		-	-		

SD: Standard deviation.

(p<0.001), +60 (p=0.002) and +90 (p=0.013). Post-stressor cortisol values differed between the alcohol-dependent patients and their relatives at timepoints +15 (p<0.001), +30 (p<0.001), +45 (p<0.001), +60 (p<0.001), and +90 (p=0.005). The ACTH levels differed between the alcohol-dependent patients and the relative and control groups (p=0.001). Post-stressor ACTH values differed between the alcohol-dependent patients and controls at timepoints +15 (p<0.001), +30 (p<0.001), +45 (p<0.001), +60 (p<0.001), and +90 (p<0.001). Post-stressor ACTH values differed between the alcohol-dependent patients and their

Table 2. Cortisol levels at rest and after stress

	Alcohol addict	Relative of addict	Control	
Cortisol	Mean±SD	Mean±SD	Mean±SD	*p*
–30	8.10±0.61[b]	8.44±0.47[b]	8.36±0.58	0.047
0	8.19±0.44[a]	8.39±0.37	8.54±0.44[a]	0.008
15	9.83±1.06[a,b]	11.40±1.88[b]	11.55±1.91[a]	<0.001
30	12.35±0.67[a,b]	15.77±1.35[b]	16.01±1.56[a]	<0.001
45	9.98±0.50[a,b]	12.61±1.38[b]	12.64±1.42[a]	<0.001
60	8.16±0.36[a,b]	8.76±0.61[b]	8.62±0.68[a]	<0.001
90	7.30±0.69[a,b]	7.64±0.33[b]	7.60±0.29[a]	0.008

SD: Standard deviation; a: The difference between alcohol addict and control is statistically significant; b: The difference between alcohol addict and relative is statistically significant; c: The difference between relative and control is statistically significant.

Table 3. Adrenocorticotropic hormone levels at rest and after stress

	Alcohol addict	Relative of addict	Control	
ACTH	Mean±SD	Mean±SD	Mean±SD	*p*
–30	12.89±0.49[a,b]	13.78±0.39[b]	13.82±0.40[a]	<0.001
0	12.99±0.54[a,b]	14.17±0.50[b]	14.11±0.44[a]	0.001
15	32.46±0.81[a,b]	37.96±0.68[b,c]	38.85±0.71[a,c]	<0.001
30	22.97±0.60[a,b]	26.59±0.49[b,c]	26.90±0.62[a,c]	<0.001
45	17.94±0.76[a,b]	19.56±0.63[b]	19.56±0.62[a]	<0.001
60	14.66±0.61[a,b]	16.16±0.72[b,c]	15.70±0.62[a,c]	<0.001
90	10.30±0.50[a,b]	12.01±0.57[b,c]	11.36±0.50[a,c]	<0.001

ACTH: Adrenocorticotropic hormone; SD: Standard deviation; a: The difference between alcohol addict and control is statistically significant; b: The difference between alcohol addict and relative is statistically significant; c: The difference between relative and control is statistically significant.

relatives at timepoints +15 (p<0.001), +30 (p<0.001), +45 (p<0.001), +60 (p<0.001), and +90 (p<0.001).

DISCUSSION

The primary aim of this study was to determine whether stress-vulnerability exists in alcohol-dependent patients and their first-degree relatives when accompanying psychiatric comorbidity is excluded; and if such vulnerability exists, whether HPA axis hypo-reactivity to the stress is a risk factor for alcohol dependence. The primary result of the study is obtaining a blunted response to psychological stress in the group of abstinent alcohol-dependent individuals for three weeks compared to the other two groups in rest and post-stress term.

A recent study also showed that alcohol-related disorders are associated with chronic changes in the HPA axis and that these patients had significant changes in serum total cortisol levels in the early morning hours.[20] Although numerous studies exist in the literature predicting blunted responses to both stress stimuli, there is evidence implying that both stress stimuli affect

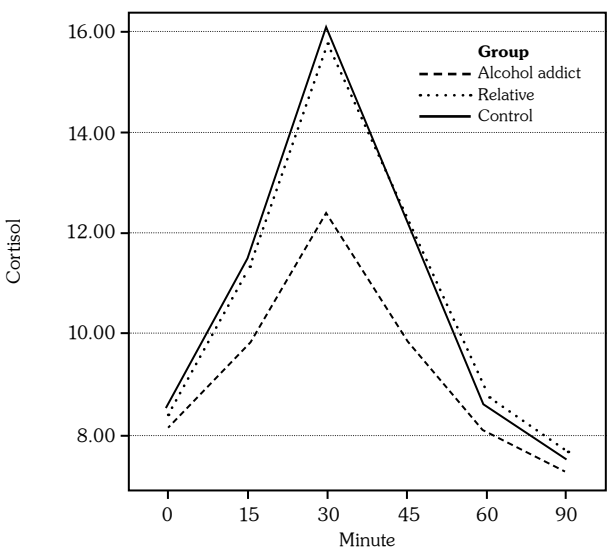

Figure 1. Cortisol values during rest and after stress in alcohol addicted patient group, unaffected first-degree relatives of patients and healthy control group.

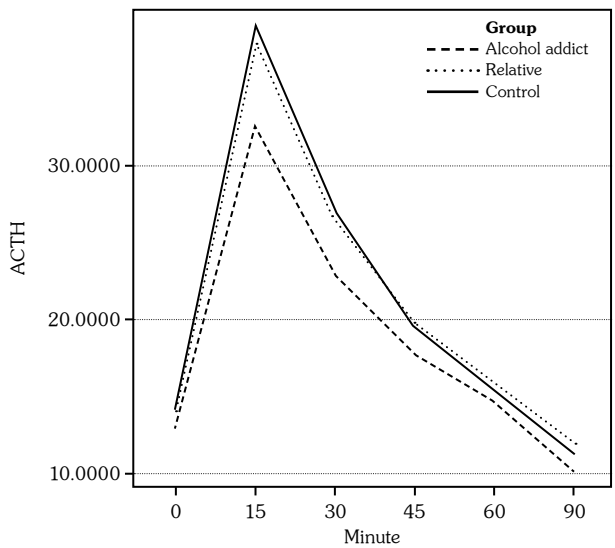

Figure 2. ACTH values during rest and after stress in alcohol addicted patient group, unaffected first-degree relatives of patients and healthy control group.
ACTH: Adrenocorticotropic hormone.

the axis in different ways. In the study performed by Lovallo et al.,[9] blunting of the HPA axis response to psychological stress was observed in the alcohol-dependent patients compared to the control group. The decrease in HPA axis response to psychological stress might be related to the moods of groups rather than the alcohol addiction. Therefore, post-traumatic stress disorder and depression were eliminated both clinically and by psychometric measurement methods during the design of the study, and salivary cortisol levels were measured.

Munro et al.[13] did not observe differences between groups for the HPA axis response to psychological stress in the addict group when compared with the control group. The patients diagnosed with comorbid axis-I affecting HPA axis activity were excluded from the study; 18 patients who were abstinent for seven days to 11 months and eight patients who were abstinent for 12 months to 17 years were included in the study. Those results contradict the results of our study. Unresponsiveness to psychological stress can be explained by a lower number of the cases included and the longer periods of abstinence. Indeed, when literature is searched in terms of the possible role of abstinence period on axis activity, many studies performed on alcohol-dependent participants.

A previous study showed a blunted adrenal response to externally applied CRH in the 1st-3rd weeks of abstinence and that the abnormality still remains in the 6th month of abstinence.[21] When the response to psychological and physical stressors is blunted, blunted adrenal responses were observed to the insulin-inducted hypoglycemia stress in patients who were abstinent for 1-2 weeks and to mental arithmetic and cold pressor test stress in patients who were abstinent for 3-4 weeks.[22] In spite of this, when HPA axis response to physical stress was investigated in the 4th, 6th, and 8th weeks of the abstinence period in a study in which alcohol-dependent patients were compared with a control group, blunting was found to be ongoing in the 4th and 6th weeks; but axis activity was found to have recovered in the 8th week. Though this finding contradicts those of the previous studies, it was hypothesized that abnormality in axis activity could recover more quickly in cases with less severity.

It is also unclear whether the duration of the abstinence period contributes to abnormality at axis activity. A significant difference between the control group and the alcohol-dependent group was not found in the study performed by Munro et al.[13] This condition might be related to the fact that patients had been abstinent from alcohol for anywhere from seven days to 17 years, which arguably created a heterogeneous group of alcohol-dependent participants. In contrast, the present study included patients who had all been abstinent for three weeks. Our findings indicate that HPA axis abnormalities and blunted ACTH/cortisol responses to stress continue through the 3rd week of abstinence. A noteworthy point is that we used stressors identical to those in the study performed by Munro et al.[13] and a mental arithmetic test for 15 min was applied following the speech against society stress for 30 min.

Physical/pharmacological/psychological stresses were applied in many of the prior studies in this area. Each type of stressor is known to stimulate the HPA axis via different mechanisms. The stimulation by physical stress is suggested to stimulate the ACTH/cortisol levels via AVP activation.[23] This situation supports that the unresponsiveness of the HPA axis is related to a disruption at the pituitary level independent of the CRH release defect. The speech against society stress, which is known to provoke anxiety and fear and strongly stimulate cortisol secretion was used in this study. Psychological stressors are the ones in which a threat perception is strongly felt. The increase in cortisol levels obtained in psychologically disturbing manipulations requires a connection between cortex, basal forebrain, amygdala, and hippocampus.[24] These responses obtained from the patient groups are thought to be dependent on the effects of excessive alcohol intake on the HPA axis or they are indications of preexisting differences in the brain areas responsible for psychosocial response generation.

The stress-induced ACTH responses were found to be significantly lower in the alcohol-dependent group at all timepoints at baseline and after the application of the stressor. The decreased activation findings observed in response to stress in the alcohol-dependent group when compared to

the first-degree relatives can be interpreted as the effect of excessive alcohol use on the HPA axis. Moreover, a statistically significant difference in terms of cortisol values with the alcohol-dependent patients and their first-degree relatives was found and was evaluated as the result of a decrease in ACTH values.

The CRH binding ability of corticotrophin cells in the pituitary gland decreased owing to chronic exposure to alcohol. Similarly, basal and CRH-stimulated AC activity and the pro-opiomelanocortin (POMC) messenger ribonucleic acid levels in the pituitary decreased even in animal studies.[25] Both the ACTH and ß-endorphine production in the anterior pituitary decreases owing to the decreasing POMC synthesis effect of alcohol. The basal levels of ACTH and ß-endorphine were found to be low in studies performed during a period of abstinence from alcohol, and this decrease is linked with the chronic effects of alcohol on the pituitary gland. Lower ACTH and cortisol levels seen in the alcohol-dependent patients at baseline might still be a reflection of the diminished stress response since exposure to a novel laboratory environment and institution of blood draws are stressful. So, the possibility of a diminished stimulation of the stress axis at the level of the hypothalamus cannot be ruled out.

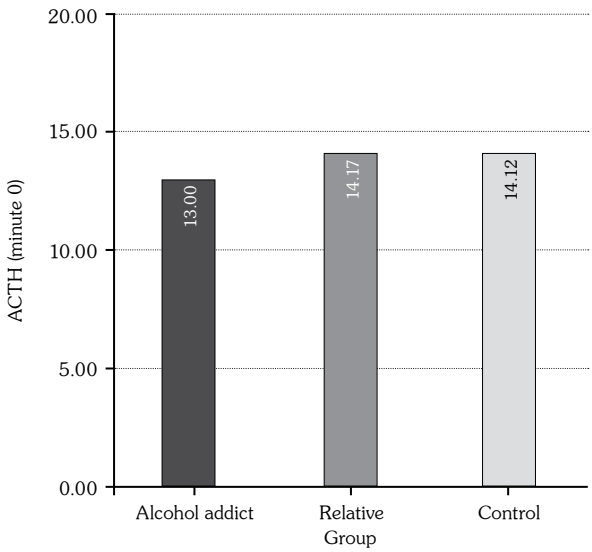

Figure 3. Basal ACTH values measured at 0 min in alcohol addicted patient group, unaffected first-degree relatives of patients and healthy control group.
ACTH: Adrenocorticotropic hormone.

In addition, the main determinants of HPA axis activity are genetic background, early-life conditions, and present life stress. Alterations in HPA axis regulation are found to be related to problematic alcohol use and dependence; however, the nature of this dysregulation seems to vary with respect to the stage of alcohol dependence. Much of the research has specifically focused on the role of cortisol in the risk for, development of, and relapse to chronic alcohol use.[26] Chronic alcohol exposure also is associated with systemic dysregulation of the HPA, sympathetic adrenal medullary system, and sex steroid systems.[27]

Furthermore, permanent alterations in HPA neuroendocrine function entailed by exposure to chronic alcohol and abstinence may also activate the brain stress systems other than the HPA axis.[28]

Even the influence of stress associated with chronic alcohol exposure on drinking has been emphasized in recent research.[29]

The HPA axis activity can be affected by psychiatric comorbid conditions. In this study, unlike other studies, first-degree relatives of alcohol-dependent patients were included in the study during the abstinence period, and all psychiatric comorbidities of the people included in the study were excluded. If the activity of the HPA axis in first-degree relatives of alcoholics shows hypoactivity after the applied stress, this situation is a sign of alcohol dependence. The presence of risk factors was assessed. The result of the study was that while there was no difference in ACTH and cortisol levels at rest, there was a statistical difference in ACTH responses in response to stress. This led to a preliminary conclusion that there is a pituitary predisposition to alcohol dependence.

In the present study, basal ACTH levels were significantly lower in alcohol-dependent patients, which is consistent with the literature (Figure 3), compared to the other two groups. This impairment in the response of the stress-axis in alcohol-dependent patients during a period of abstinence, but not in their first-degree relatives or controls, suggests that the impairment may be a pre-existing risk factor for alcohol dependence.

The most important limitation of our study was the small sample groups. Further studies with larger sample sizes are needed to fully address these questions regarding the effects of chronic alcohol on the pituitary level. Based on the evaluations conducted in this study, a hypothesis was formed regarding a potential predisposition at the pituitary level, and a consensus was reached on the necessity of further research with larger sample sizes.

In conclusion, in our study we investigated whether stress-vulnerability exists in alcohol-dependent patients and their first-degree relatives; and if such vulnerability exists, whether HPA axis hyporeactivity to the stress is a risk factor for alcohol dependence. We found a deficient activation of the HPA axis in recently abstinent alcohol-dependent patients than in their first-degree relatives and controls.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, design, control/supervision, data collection and/or processing, analysis and/or interpretation, writing the article, references and fundings, materials: E.K.K.; Control/supervision,analysis and/or interpretation,literature review,writing the article, critical review: B.H.A.; Idea/concept, design, control/supervision, critical review, references and fundings: N.D.; Analysis and/or interpretation, literature review, data collection and/or processing: N.A.K.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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