Original article / *Araştırma*

Plasma allopregnanolone levels in drug-free, comorbidity-free obsessive-compulsive disorder

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ABSTRACT

Objective: *Although there are studies in the literature focusing on the relationship between neurosteroids and psychiatric disorders, the studies on patients with obsessive-compulsive disorder (OCD) are limited in number. Nevertheless, allopregnanolone, a neurosteroid, in OCD patients has not been investigated in this limited number of studies. Allopregnanolone is considered to have a role in the pathogenesis of many psychiatric disorders. Accordingly, the present study aimed to investigate the relationship between the neurosteroid allopregnanolone and OCD.* ***Methods:*** *The study included 40 OCD patients diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) diagnostic criteria and 40 healthy control subjects. The study participants completed the Yale-Brown Obsessive Compulsive Scale. Taking the diurnal rhythm of neurosteroids into consideration, blood samples for determination of plasma allopregnanolone levels were obtained from all patients and controls between 09:00 and 10:00 in the morning after 12 hours of fasting, tobacco abstinence, and 30 minutes of resting period. Plasma allopregnanolone level was analyzed using the enzyme-linked immunosorbent assay (ELISA).* ***Results:*** *No significant difference was determined between the patient and control groups regarding serum allopregnanolone levels. Evaluation of the allopregnanolone levels of the patients for each obsession type according to its presence and absence revealed no significant difference in any of them. Evaluation of the allopregnanolone levels of the patients for each compulsion type according to its presence and absence revealed that the allopregnanolone level of the patients with counting-organizing compulsive behavior was lower than that of those without.* ***Discussion:*** *Although there was no difference between OCD patients and controls regarding plasma allopregnanolone level, it is difficult to make a conclusion that allopregnanolone level does not play a role in the etiopathogenesis of disease. Conducting further studies, which would analyze allopregnanolone level in materials other than plasma such as cerebrospinal fluid using multiple analyses and separately evaluates females and males with higher number of patients, is of importance to enlighten at least some aspects of allopregnanolone-OCD relationship. Moreover, significantly lower plasma allopregnanolone level particularly in a single type of compulsion brings in mind again the suggestion that different etiopathogenesis might have a role in the symptom subtypes of OCD, which has begun to be discussed in the recent years.* ***(Anatolian Journal of Psychiatry 2018; 19(5):435-442)***

Keywords: *obsessive-compulsive disorder, allopregnanolone, neurosteroids*

İlaç kullanmayan ve eş tanısı olmayan obsesif kompulsif bozukluk hastalarında plasma allopregnanolon düzeyleri

ÖZ

Amaç: *Literatürde nörosteroid ve psikiyatrik bozuklukların ilişkisine yönelik çalışmalar olsa da, obsesif kompulsif bozukluk (OKB) hastalarında yapılmış az sayıda çalışma vardır. Çalışmalar incelendiğinde OKB hastalarında bir nörosteroid olan allopregnanolon düzeylerinin araştırılmadığı görülmektedir. Allopregnanolonun birçok psikiyatrik*

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bozukluğun patogenezinde etkisinin olduğu düşünülmektedir. Biz de çalışmamızda bir nörosteroid olan allopregnanolon ve OKB arasındaki ilişkiyi incelemeyi amaçladık. Çalışmamızın bulgularının bozukluğun etiyopatogenezinin aydınlatılmasına ışık tutacağını ve belki de bu konunun aydınlatılmasına katkıda bulunacak yeni çalışmalarla beraber potansiyel yeni tedavi hedefleri belirlenmesine katkı sağlayacağını düşünmekteyiz. ***Yöntem:*** *Çalışmaya DSM-5 tanı ölçütlerine göre 40 OKB hastası ve 40 sağlıklı gönüllü alındı. Katılımcılara Beck Depresyon Ölçeği, Beck Anksiyete Değerlendirme Ölçeği ve Yale-Brown Obsesyon Kompulsiyon Değerlendirme Ölçeği uygulandı. Allopregnanolon için kan örnekleri hasta ve kontrol gruplarından nörosteroidlerin diurnal ritmi olduğu düşünülerek sabah 09:00-10:00 arasında, 12 saatlik açlık, tütün perhizi ve 30 dakikalık dinlenme sonrası alındı. Plasma allopregnanolon düzeyi ELISA yöntemiyle analiz edildi.* ***Bulgular:*** *Allopregnanolon düzeyleri açısından gruplar arasında istatistiksel olarak anlamlı bir fark saptanmadı. OKB belirti alt tipleri kendi içinde incelendiğinde obsesyon türleri arasında allopregnanolon düzeyleri açısından anlamlı fark saptanmazken, kompulsiyon türlerinden sayma ve düzen kompulsiyonları olanlarda olmayanlara göre allopregnanolon düzeylerinin istatistiksel olarak anlamlı derecede düşük olduğu görüldü.* ***Tartışma:*** *Çalışmamızda OKB hastaları ve kontrol grubu arasında allopregnanolon düzeyleri açısından fark gözlenmemiş olsa da, tam olarak bozukluğun etiyopatogenezine katılmadığını söylemek zordur. Erkek ve kadınların ayrı değerlendirildiği, daha fazla hasta ile, plazma dışında BOS gibi farklı materyallerden çoklu analizlerle yapılacak çalışmalar, allopregnanolon-OKB ilişkisinin bazı yönlerinin aydınlatılması açısından önemli görünmektedir. Ayrıca sayma-düzen kompulsiyonu olanlarda olmayanlara göre allopregnanolon düzeylerinin anlamlı derecede düşük olması bu konuda daha önce yapılan hayvan çalışmasını desteklemektedir. Ayrıca kompulsif belirtilerden sadece bir kompulsiyon tipinde anlamlı düşüklük gözlenmesi özellikle son yıllarda tartışılmaya başlanan OKB'nin belirti alt tiplerinde farklı etiyopatogenezlerin rolü olabileceği düşüncesini desteklemektedir.* ***(Anadolu Psikiyatri Derg 2018; 19(5):435-442)***

Anahtar sözcükler: *Obsesif-kompulsif bozukluk, allopregnanolon, nörosteroidler*

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a common psychiatric disorder that significantly influences functionality of individuals. Neurobiological factors increasingly gain importance in the etiology of OCD.[1,2] The studies focusing on the relationship between OCD and neurosteroids have found the levels of dehydroepiandrosterone (DHEA) and cortisol to be higher in the OCD patients than in the controls.[1]

In the literature, neurosteroids are reported to have significant modulating effects on brain functions,[2] and to be potentially associated with the pathophysiology of neuropsychiatric diseases such as premenstrual syndrome, epilepsy, schizophrenia, anxiety, depression, alcohol addiction, multiple sclerosis and OCD.[1,3-5] A limited number of studies investigating the relationship between OCD and neurosteroids has not been investigated allopregnanolone, a neurosteroid, in OCD patients. In recent years, allopregnanolone has been thought be involved in the pathogenesis of many psychiatric disorders. Allopregnanolone is a positive modulator of gamma aminobutyric acid-A (GABA-A), which is the main inhibitor system of the brain. Particularly in the last decade, the studies investigating anxiolytic, antidepressant, anticonvulsant, antistress, and behavioral effects of allopregnanolone on the glutamate receptors due allosteric modulation and neuronal plasticity mediated by GABA-A and N-methyl-D-aspartate receptors have increased in number.[6,7] In the literature, there are numerous studies indicating that depression, anxiety disorders, premenstrual dysphoric syndrome, and schizophrenia may influence serum allopregnanolone levels.[8-14] Nevertheless, there is a single study investigating the relationship between allopregnanolone and OCD, which demonstrated that allopregnanolone could affect compulsive behavior.[15] In that particular study, marble-burying behavior of rodents was accepted as the compulsive component of OCD and allopregnanolone injection was observed to block marble-burying behavior. This effect resembles that of fluoxetine, a selective serotonin reuptake inhibitor (SSRI) used in the treatment of OCD.[15]

In this study, we aimed to investigate the role of allopregnanolone in the pathogenesis of OCD by comparing plasma allopregnanolone levels between OCD patients and healthy control subjects.

METHODS

The present study included 40 drug-naive patients aged 18-65 years who were admitted to the Psychiatry clinic of Inonu University and diagnosed with OCD based on the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) diagnostic criteria and gender-age matched 40 healthy subjects as a control group (CG). The diagnosis was established based on the Structured Clinical Interview for DSM-IV-TR/Clinical Version (SCID-I/CV) by a senior psychiatry intern and a psychiatrist inde-

pendent from each other through an interview with the patients. Exclusion criteria for the patient group (PG) and CG were as follows: presence of major systemic disease, endocrine pathology, neurological disease, a previous head trauma, alcohol/substance addiction, having undergone electroconvulsive therapy, having schizophrenia or other psychotic disorder in the past or during interview, experiencing a manic or hypomanic episode, having mental retardation, being pregnant or being in lactation period, and receiving oral contraceptive agent. CG consisted of age-gender matched 40 healthy subjects. The study protocol was approved by the Local Ethics Committee of Medical Faculty of İnönü University. The study was carried out in accordance with the principles of 'Declaration of Helsinki for Human Rights-2001 version' and 'Good Clinical Practices'. Written consents of the patients were obtained after informing them about the study objective and methods.

The Yale-Brown Obsessive Compulsive Scale (Y-BOCS) was performed to evaluate disease severity in the patients. Beck Depression Inventory and Beck Anxiety Inventory performed. Taking the diurnal rhythm of neurosteroids into consideration, blood samples for determination of plasma allopregnanolone levels were obtained from all patients and controls between 09:00 and 10:00 in the morning after 12 hours of fasting, tobacco abstinence and 30 minutes of resting period. Blood samples were centrifuged at 3500 rpm for 10 minutes. After centrifugation, the serums were separated into fractions and stored at -80ºC. Analysis of plasma allopregnanolone levels was performed by the enzyme-linked immunosorbent assay (ELISA) using Elabscience Human AP ELISA kit (Elabscience Biotechnology Co., Ltd., China). Anti-human allopregnanolone antibodies are adsorbed to the micro-plate wells. Allopregnanolone in the sample or in the standard binds to the antibodies adsorbed to the wells. Biotin-conjugated allopregnanolone antibodies bind to allopregnanolone, which is conjugated with the initial antibody. Thereafter, unbound antibodies are removed by washing procedure, streptavidin-HRP is added into the media, and this complex binds to the biotin-conjugated anti-human allopregnanolone antibodies. Substrate and color reactive are added into the media. H_2O_2 is used as the substrate and the mixture of 4-aminoantipirin and phenol is used as the color reactive. The reaction is terminated with acid added into the media and the level of the associated parameter is determined with measurement of the absorbance of colorful reactive at 450 nm. For determining the allopregnanolone level, the standards were prepared at the concentrations of 100, 50, 25, 12.5, 6.25, 156.3, 3.125, 1.563, and 0 ng/mL and a standard graphic was established.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 17.0, SPSS, Inc., Chicago, Il, USA) for Windows. Data were expressed as mean and standard deviation (SD) for sociodemographic variables and as median (minimum-maximum) and number (percentage) for other variables. Normality of data was assessed by the Shapiro-Wilk test. For the comparisons of the groups, t-test was used for independent analysis of the normally distributed variables and Mann Whitney U test was used for the non-normally distributed variables. Correlation analysis was performed using Pearson's correlation test for normally distributed variables and using Spearman's correlation test for non-normally distributed variables. A p value of <0.05 was considered statistically significant.

RESULTS

The general characteristics of the PG and CG are presented in Table 1. The mean age was 32.82±9 years both in the PG and CG. Comparison of the general characteristics between the two groups revealed no significant difference in terms of sociodemographic variables ($p>0.05$ for all) (Table 1).

In the PG, the median Y-BOCS total score was 16 (range: 8-35), the median Y-BOCS obsession score was 9 (range: 5-19), and the median Y-BOCS compulsion score was 7.5 (range: 2-17). For the female patients, the median Y-BOCS total score was 16 (range: 9-35), the median Y-BOCS obsession score was 9.5 (range: 5-19), and the median Y-BOCS compulsion score was 8 (range: 2-17). For the male patients, the median Y-BOCS total score was 15.5 (range: 8-34), the median Y-BOCS obsession score was 9 (range: 5-19), the median Y-BOCS compulsion score was 7 (range: 3-15) (Table 2).

The median allopregnanolone level was 76.43 ng/mL (range: 31.54-87.74 ng/mL) in the PG and 71.46 ng/mL (range: 12.44-89.94ng/mL) in the control group. Comparison of the plasma allopregnanolone level between the two groups revealed no significant difference ($p=0.254$) (Table 3).

Table 1. General characteristics of the study groups

Characteristics	Patient group (n=40) n	%	Control group (n=40) n	%	p
Age, (Mean±SD, years)	32.82±9		32.82±9		>0.05
Gender					>0.05
Male	18	45.0	18	45.0	
Female	22	55.0	22	55.0	
Marital status					>0.05
Single	14	35.0	9	22.5	
Married	25	62.5	27	67.5	
Divorced	1	2.5	4	10.0	
Education level					>0.05
Primary school	7	17.5	0	0	
High school	7	17.5	9	22.5	
College/university	26	65.0	31	77.5	
Smoking					>0.05
Yes	15	37.5	8	20.0	
No	25	62.5	32	80.0	
Alcohol consumption					>0.05
Yes	4	10.0	3	7.5	
No	36	90.0	37	92.5	
BMI (Mean±SD, kg/m^2)	24.31±2.93		24.63±3.25		>0.05
Disease duration (Mean±SD, y)	9.5±7.5				

BMI: Body Mass Index

Table 2. Yale-Brown Obsessive Compulsive Scale scores of the patient group

Y-BOCS	All patients (n=40) Median	min-max	Female patients (n=22) Median	min-max	Male patients (n=18) Median	min-max
Obsession subscale score	9	5-19	9.5	5-19	9	5-19
Compulsion subscale score	7.5	2-17	8	2-17	7	3-15
Total score	16	8-35	16	9-35	15.5	8-34

Y-BOCS: Yale-Brown Obsessive Compulsive Scale

Table 3. Comparison of allopregnanolone levels between groups

Groups	Allopregnanolone level (ng/mL) Median	min-max	Groups	Allopregnanolone level (ng/mL) Median	min-max	p
Patients (n=40)	76.43	31.54-87.74	Controls (n=40)	71.46	12.44-89.94	0.254
Female patients (n=22)	77.74	34.38-87.28	Female controls (n=22)	74.05	57.98-89.94	0.734
Male patients (n=18)	73.94	31.54-87.74	Male controls (n=18)	64.61	12.44-83.09	0.184

Considering that allopregnanolone level can be influenced by gender, the groups were divided into gender subgroups. Comparison of the median allopregnanolone level of the female patients with that of female controls revealed no significant difference (77.74 ng/mL [range: 34.38-87.28 ng/mL] and 74.05 ng/mL [range: 57.98-89.94 ng/mL], p>0.05) (Table 3). Comparison of the median allopregnanolone level of the male patients with that of male controls revealed no significant difference (73.94 ng/mL [range: 31.54-87.74 ng/mL] and 64.61 ng/mL [range: 12.44-83.09 ng/mL], p>0.05) (Table 3).

Table 4. Evaluation of allopregnanolone levels of the patients for each obsession type

Obsession types	Allopregnanolone level (ng/mL) Median	min-max	p
Aggression			0.744
Present	76.43	31.54-87.28	
Absent	74.81	34.38-87.74	
Contamination			0.553
Present	76.26	34.38-87.28	
Absent	81.76	31.54-87.74	
Sexual			0.698
Present	79.78	60.37-83.74	
Absent	76.26	31.54-87.74	
Hoarding			
Present	-	-	-
Absent	76.43	31.54-87.74	
Religious			0.956
Present	75.04	51.90-86.49	
Absent	77.74	31.54-87.74	
Symmetry-order			0.639
Present	75.04	34.38-87.28	
Absent	79.04	31.54-87.74	
Somatic			0.526
Present	76.61	67.73-83.74	
Absent	76.26	31.54-87.74	
Other			0.837
Present	73.81	31.54-87.74	
Absent	76.94	34.38-87.28	

Table 5. Evaluation of allopregnanolone levels of the patients for each compulsion type

Compulsion types	Allopregnanolone level (ng/mL) Median	min-max	p
Cleaning			0.512
Present	76.77	34.38-87.28	
Absent	67.60	31.54-87.74	
Ritual			1.00
Present	72.71	42.30-86.49	
Absent	76.77	31.54-87.74	
Counting-organizing			0.034
Present	55.76	34.38-76.95	
Absent	77.57	31.54-87.74	
Other			0.786
Present	76.43	31.54-87.74	
Absent	74.81	34.38-87.28	

In the PG, correlation analysis performed to evaluate the relationship of serum allopregnanolone level with disease duration, total, obsession, and compulsion scores of the YBOCS revealed no significant correlation ($p>0.05$).

In the study, allopregnanolone levels of the patients were evaluated for each obsession type according to its presence and absence; no significant differences were found ($p<0.05$ for each) (Table 4). Evaluation of the allopregnanolone levels of the patients for each compulsion type according to its presence and absence revealed that the median allopregnanolone level of the patients with counting-organizing compulsive behavior were lower than that of those without ($p=0.034$) (Table 5).

DISCUSSION

The first outcome of this study was the lack of a significant difference between the OCD patients and controls in terms of serum allopregnanolone level. Second outcome was the allopregnanolone level being significantly lower in the patients with counting-organizing behavior than those without. To our knowledge, there is a single study focusing on the relationship between allopregnanolone and OCD and demonstrating that allopregnanolone could affect compulsive behavior. In that study conducted in 2009 by Umathe et al.,[15] the marble-burying behavior of the rodents was considered as the compulsive component of the OCD and it was blocked by allopregnanolone injection. This effect was found to be similar to that of fluoxetine and the effects of allopregnanolone and fluoxetine were also compared.[15]

The outcomes of this study were consistent with the results of the study by Umathe et al.[15] The action mechanism of neurosteroids on compulsive behaviors such as marble-burying could not been clearly explained; however, underlying mechanisms may include the fact that allopregnanolone reduces dopamine neurotransmission and elevates triggering threshold of serotonergic neurons in the raphe nucleus by inhibiting dopamine release in the basal ganglia, as well as GABA-A receptor modulation.[15-17] Another study focusing on the neurosteroid-OCD relationship has reported a positive correlation between serum DHEA level and severity of compulsion;[1] this was the first report about the relation of neurosteroids with compulsions in humans. From this point of view, given that the underlying factor for compulsive behaviors is low allopregnanolone level; it is replacement might be a new treatment target in the future. Further studies

on this field would contribute to the illumination of this subject. Considering that the serum allopregnanolone level might reflect the allopregnanolone level in the brain, the relation of this finding with the disease makes more sense, due to the significant correlation between serum and cerebrospinal fluid (CSF) levels of neurosteroids.[18]

Clinical trials investigating the relationship between allopregnanolone and anxiety disorders have reported that allopregnanolone level remains unchanged in generalized anxiety disorder, social phobia, and mixed anxiety-depressive disorder.[10,11,13] In the study, the lack of a significant difference between the OCD patients and controls in terms of allopregnanolone levels appears to support this finding. Since OCD has been among the anxiety disorders until the introduction of DSM-5 diagnostic criteria and OCD patients have to compete with the anxiety arising from their obsessions, our finding is important by providing information about the role of allopregnanolone in the etiology of anxiety.

In panic disorder, however, there are different outcomes in the literature concerning menstrual period and the agent triggering the attacks. This could be explained rather by the relation of allopregnanolone with the triggering factor than its role in the etiopathogenesis of panic attack.[5,19-21] Nevertheless, a linear relation could not be demonstrated between the symptom severity and allopregnanolone level. Low allopregnanolone levels have been determined in those with severe symptoms of anxiety and irritability. This is explained by biphasic effect of allopregnanolone as is in numerous GABA-A receptor modulators. In other words, it shows anxiogenic action in low concentrations but anxiolytic action in high concentrations.[22,23]

Another data retrieved from the studies focusing on panic disorder have indicated that allopregnanolone level is low during menopausal period but high in early follicular phase in panic attack patients.[5,24] Significant increases have been determined in allopregnanolone level and allopregnanolone/progesterone ratio in the luteal phase in women with premenstrual dysphoric syndrome. One of the limitations of the study was the fact that blood samples of females were not collected during the mid-luteal phase.

As allopregnanolone level varies according to gender, we evaluate study groups by regrouping them as females and males. Accordingly, the allopregnanolone level showed no significant difference between the male patients and controls as well as between the female patients and controls.

In the present study, taking the diurnal rhythm of neurosteroids into consideration, blood samples were collected from all patients and controls between 09:00 and 10:00 in the morning after 12 hours of fasting and tobacco abstinence period; however, seasonal changes in neurosteroids were not taken into account, which could be considered as the other limitation of the present study.

Neurosteroids have critical role in early neuronal development due particularly to their effects in GABA-A receptor modulation.[25] Although allopregnanolone level increases in order to reduce the hypothalamic-pituitary-adrenal axis activetion, which is elevated in response to acute stress, low allopregnanolone level is deemed effective in diseases such as depression and anxiety following long-term stress.[14] An increase in allopregnanolone level during acute stress is considered as an endogenous protective factor. Assuming that allopregnanolone increases in the acute phase as a compensatory factor to reduce anxiety, it can be thought that it decreases back to its normal range with the prolongation of the disease. Further studies comparing different patient groups, which are in different phases of the disease, are required to enlighten this subject.

It is known that allopregnanolone levels are influenced by numerous drugs and physical diseases since allopregnanolone is a neurosteroid. In the studies on depression and schizophrenia, significant increases have been observed in the brain allopregnanolone level after treatment with antidepressants such as fluoxetine, norfluoxetine, fluvoxamine, and paroxetine and with antipsychotics and/or mood stabilizers such as carbamazepine.[26-28] For this reason, in the present study, patients who did not receive any psychiatric drug and/or any drug likely to influence neurosteroid levels in the last six months were enrolled. This makes the data of the present study valuable. In addition, the lack of a significant difference between the patient and control groups in terms of cigarette-tobacco use, alcohol consumption, and BMI was one of the strengths of the present study.

In the literature, there are numerous studies indicating that depression and anxiety disorders may influence allopregnanolone levels.[8-14] For this reason, the study participants with accompanying major depressive disorder or anxiety disorder, of which the diagnosis was established by a senior psychiatry intern and a psychiatrist

independently of each other via an interview before patient enrollment, were excluded. The fact that only the patients with pure OCD were enrolled in the present study strengthens the outcomes.

Evaluation of the allopregnanolone levels of the patients for each obsession symptoms according to its presence and absence revealed no significant difference. On the other hand, among compulsive symptoms, plasma allopregnanolone level was found to be significantly lower in the patients with counting-organizing compulsive behavior than in those without. This finding was consistent with the results of the unique study[15] in the literature investigating the relationship of OCD and allopregnanolone. Determining a significantly lower level of allopregnanolone level in only a single type of compulsive behavior but not in the others again suggests that different etiopathogenesis might have a role in the symptom subtypes of compulsion, which has particularly begun to be discussed in the recent years. Recently, the studies focusing on the hypothesis that OCD subtypes such as washing and controlling and diverse symptom clusters may be associated with different etiology, different dysfunction or different neuronal pathways have increased in number.[29,30]

In conclusion, based on the results of the present study, it is difficult to suggest that allopregnanolone does not involve in the etiopathogenesis of OCD. However, conducting further studies, which would analyze allopregnanolone level in materials other than plasma such as CSF using multiple analyses and separately evaluates females and males with higher number of patients, is of importance to enlighten at least some aspects of allopregnanolone-OCD relationship. To our knowledge, the literature has no study conducted on this subject; for this reason, we think that the result of the present study would contribute to the literature on this subject and be helpful in determining new potential treatment targets together with further future studies.

Authors' contributions: *L.U.İ.: literature review, sample collection, applying scales, writing the manuscript; L.G.E.: finding the subject, conducting research, review the manuscript.*

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