Article

Discovery Medicine 2024; 36(190): 2244–2252
https://doi.org/10.24976/Discov.Med.202436190.206

Bipolar Disorder and Ubiquitin Proteasome System Dysfunction: Peripheral Blood Levels of Molecules Playing a Role in Ubiquitination and Their Relationship to Sleep Quality

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Published: 20 November 2024

Background: Bipolar disorder (BD) is a serious mood disorder, notable for its morbidity and prevalence. It ranks among the top 10 diseases globally in terms of functional impairment among affected individuals. Studies investigating neurobiological processes in the development of BD also aim to identify biological markers. Ubiquitin is a protein that is abundant in all eukaryotic cells and regulates many processes through the ubiquitin-proteasome system. It has been reported to be associated with circadian rhythm and sleep disorders. Circadian rhythm plays a key role in maintaining mood stability in individuals with BD. In this study, we investigated the peripheral levels of molecules involved in the ubiquitination process and their relationship to sleep quality in individuals with BD.

Methods: Forty-nine patients with BD and 50 healthy volunteers without any psychiatric disorders were included. The Pittsburgh Sleep Quality Index, the Young Mania Rating Scale, and the Hamilton Depression Rating Scale were administered to the participants. Peripheral blood levels of proteins and enzymes that play a role in ubiquitination processes were determined by the immunosorbent assay method.

Results: TAR DNA-binding protein-43 (TDP-43) ($p < 0.001$), ubiquitin C-terminal hydrolase-L1 enzyme (UCH-L1) ($p = 0.037$), ubiquitin C-terminal hydrolase-L3 enzyme (UCH-L3) ($p = 0.007$), histone deacetylase I (Histone Dea-1) ($p = 0.006$), histone deacetylase II (Histone Dea-2) ($p = 0.047$), and ligase cullin-3 ($p = 0.031$) levels were found to be significantly lower in the BD group than in the control group, but these parameters were not associated with sleep quality scores in the BD group.

Conclusions: Our results support the data in the literature but show that the ubiquitination process can be affected in BD patients without being associated with sleep quality.

Keywords: bipolar disorder; sleep quality; ubiquitination

Introduction

Bipolar disorder (BD) is a serious mood disorder characterized by manic or depressive episodes. Including its subtypes, it has been reported that its prevalence in the community may reach approximately 3% [1]. Since it is a recurrent chronic mental illness, it is prominent in terms of its morbidity and mortality. It is one of the top ten diseases in the world that causes the most loss of functionality in individuals diagnosed with the disease [2,3]. It has been stated that studies investigating neurobiological processes in the development of BD also aim to identify biological markers that could contribute to early and definitive diagnosis of the disease, make evidence-based treatment plans, and evaluate treatment response [4].

Ubiquitin is a protein that is abundant in all eukaryotic cells and regulates many events through the ubiquitin-proteasome system (UPS). UPS, along with the lysosomal system, is one of the two main proteolytic systems responsible for intracellular protein turnover. In UPS, ubiquitin molecules bind to abnormal proteins that need to be broken down and form a polyubiquitin chain. Thus, by marking these proteins, they introduce them to the proteasome, the proteolytic component of the system, for degradation. The proteasome is a multicatalytic enzymatic complex and enables rapid degradation of the target substrates. This type of protein modification is called ubiquitination [5,6].

Ubiquitin ligase, one of the molecules participating in the ubiquitination process, is the most important substrate recognition factor of the system [7]. Cullin-3 protein, on the other hand, has a critical role as the core component of the

ubiquitin ligase complex [8]. TAR DNA-binding protein-43 (TDP-43) is a nuclear protein that binds to the mRNA of ubiquitin ligase to regulate the transcription and expression of thousands of genes [9]. It has been suggested that TDP-43 mediates TDP-43 translocation by forming multiprotein complexes with histone deacetylase-6 [10]. Ubiquitin C-terminal hydrolase-L1 enzyme (UCH-L1) is involved in the removal of ubiquitin from metabolized proteins in the ubiquitin proteasome pathway [11]. It is important in providing axonal stability in the central nervous system. In the UCH-L1 deficient mice model, the level of free ubiquitin in the brain decreases by 20–30% [12]. UCH-L1 is found in nearly all neurons and accounts for an average of 1–5% of total soluble brain proteins. The abundance of UCH-L1 in the brain, compared to its limited presence in other tissues, has led to its use as a biomarker [13].

Studies have shown that the ubiquitination process is closely related to sleep and circadian rhythm regulation [8,14]. Mice with spontaneous deletion of the *UCH-L1* gene were found to have disruptions in sleep/wake rhythms compared to wild-type mice. It was also reported that ubiquitin ligase and cullin-3 played an important role in sleep and circadian rhythm through ubiquitination [14].

The circadian rhythm is the key factor in maintaining a constant sleep-wake cycle. Because of this feature, it plays a major role in maintaining mood stability in individuals with BD [15]. Many studies have shown that circadian rhythm disorders are detected in patients with BD, independent of mood episodes, and the regularity of the circadian rhythm positively affects the course of the disease [16–18]. In this study, we investigated the peripheral levels of TDP-43, UCH-L1, ubiquitin C-terminal hydrolase-L3 enzyme (UCH-L3), ubiquitin ligase, cullin-3, histone deacetylase I (Histone Dea-1), and histone deacetylase II (Histone Dea-2), molecules associated with the ubiquitination process which has a role in circadian system regulation, in individuals with BD and aimed to determine their relationship to sleep quality.

Materials and Methods

Participants

This study was carried out in the Community Mental Health Center of the Hitit University Erol Olçok Research and Training Hospital. The inclusion criteria for the patient group include being diagnosed with BD according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5, being in remission, and being 18–65 years old. The inclusion criteria for the healthy control group were not having any psychiatric disorders according to the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), being 18–65 years old, and voluntarily agreeing to participate in the study. The experimental protocol was established according to all applicable ethical guidelines and recommendations of the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (B.30.2.ODM.0.20.08/112-270). Forty-nine participants diagnosed with BD and 50 healthy volunteers of similar age and sex distributions whose written informed consent was obtained were included in the study. Various sociodemographic characteristics of the patients, including age, sex, and marital status, were recorded. The Young Mania Rating Scale (YMRS), the Hamilton Depression Rating Scale (HAM-D), and the Pittsburgh Sleep Quality Index (PSQI) were administered to the participants. For both groups, individuals who had any additional disease that would impair their judgment, such as mental retardation or organic mental disorder, and those who were experiencing manic or depressive episodes during the evaluation were excluded from the study.

Data Collection Instruments

Sociodemographic Information Form

The researchers prepared the form, which contained questions about age, place of residence, marital status, cohabitants, education, employment status, disease history, mood stabilizer usage characteristics, smoking status, and alcohol consumption status.

Pittsburgh Sleep Quality Index

PSQI is a questionnaire that evaluates sleep quality and disturbance in the last month. It was developed by Buysse *et al*. [19]. The Turkish validity and reliability study of PSQI was performed by Agargun [20]. The scale consists of 24 items, 19 of which are self-report items, while 5 are answered by the partner or roommate. With 19 questions answered by the individual, 7 dimensions are evaluated, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction. Each component is evaluated over 0–3 points. The total score of the 7 components gives the total score of the index, which ranges from 0 to 21. A total score greater than 5 indicates "poor sleep quality". The Cronbach's α coefficient of PSQI in this study was found to be 0.80.

Young Mania Rating Scale

YMRS was developed by Young *et al*. [21]. The validity and reliability study of the scale in Türkiye was carried out by Karadağ *et al*. [22]. It consists of 11 items, and each item measures symptom severity at 5 stages. The items in the scale evaluate the defined core symptoms of a manic episode of BD. The grading of severity is based on the patient's reports of their subjective condition in the last 48 hours and the clinician's impressions of the patient during the interview. The Cronbach's α coefficient of YMRS in this study was found to be 0.79.

Table 1. Comparison of age and sex of patients in BD group (n: 49) and control group (n: 50).

	Healthy control group (n: 50)	BD group (n: 49)	*p*-value
	Mean ± SD/n - %	Mean ± SD/n - %	
Age (years)	42.8 ± 8.8	44.0 ± 12.4	0.587 ^{t} (t = 0.586)
Sex			
Female	24–48.0%	25–51.0%	0.764 $^{\chi 2}$ (χ^2 = 0.090)
Male	26–52.0%	24–49.0%	

^{t}Independent-samples t-test; $^{\chi 2}$ Chi-squared test; values are presented as mean ± standard deviation or frequency (%). BD, bipolar disorder; SD, standard deviation.

Hamilton Depression Rating Scale

HAM-D was developed by Hamilton [23]. The Turkish validity and reliability study of the scale was performed by Akdemir *et al*. [24]. It is used to measure the severity of depressive symptoms. On the scale applied by the clinician, each of the seventeen items is scored between 0 and 4. The total score on the scale varies between 0 and 53. A higher total score indicates a higher severity of depression. The Cronbach's α coefficient of HAM-D in this study was found to be 0.75.

Sample Collection

Blood samples were taken into tubes with a clot activator for serum between 08.00 and 10.00 following 12 hours of fasting. After keeping the samples at room temperature for half an hour, the samples were centrifuged at 4000 rpm for 10 minutes, and the separated sera were stored at –70 °C until the analyses.

Molecular Measurements

Neuroprotective and neurodegenerative protein and enzyme levels that play a role in ubiquitination processes were determined by the enzyme-linked immunosorbent assay (ELISA) method based on the double-antibody sandwich method. Samples containing TDP-43, UCH-L1, UCH-L3, ubiquitin ligase cullin-3, cullin-3, Histone Dea-1, and Histone Dea-2 were added to plate wells coated with monoclonal antibody and incubated. After incubation, an immune complex was formed with biotin-labeled TDP-43, UCH-L1, UCH-L3, ubiquitin ligase cullin-3, cullin-3, Histone Dea-1, and Histone Dea-2 antibody and streptavidin-HRP solution. Unbound proteins and enzymes were cleaned from the medium by washing, chromogenic reagent A and B solutions were added, and the mix was incubated at 37 °C for about 10 minutes away from light. After incubation, the color change was inhibited by adding the stop solution, and the optical density of the standard and samples were determined at a wavelength of 450 nm within 10 minutes. Protein levels in the sample were determined using the optical density and concentration values of the standards.

ELISA Kits Used for Molecular Measurements

(1) TDP-43, Catalog Number: E6246Hu, Shanghai Korain Biotech, Shanghai, China.

(2) UCH-L1, Catalog Number: E2328Hu, Shanghai Korain Biotech, Shanghai, China.

(3) UCH-L3, Catalog Number: E6969Hu, Shanghai Korain Biotech, Shanghai, China.

(4) Ubiquitin ligase cullin-3, Catalog Number: E6435Hu, Shanghai Korain Biotech, Shanghai, China.

(5) Cullin-3, Catalog Number: E6968Hu, Shanghai Korain Biotech, Shanghai, China.

(6) Histone Dea-1, Catalog Number: E2041Hu, Shanghai Korain Biotech, Shanghai, China.

(7) Histone Dea-2, Catalog Number: E2025Hu, Shanghai Korain Biotech, Shanghai, China.

Statistical Method

Hitit University licensed SPSS 27.0 (IBM SPSS Inc., Chicago, IL, USA) program was used for the statistical analyses. The normality of the distributions of the variables was measured with the Kolmogorov-Smirnov test. In the descriptive statistics of the data, frequency and ratio values were used. Mean ± standard deviation or median (25th–75th quartiles) was used as appropriate for the continuous variables. Categorical variables expressed as frequencies and percentages. Independent-sample t-tests and the Mann-Whitney U-test were used for the comparisons of the normally and non-normally distributed continuous parameters, respectively. Chi-squared tests were used in the analyses of the qualitative independent data. Spearman's correlation analysis was used to test correlations. A significance level of $p < 0.05$ was considered statistically significant.

Results

Participants Features

A total of 99 individuals, 49 in the BD group and 50 in the healthy control group, were included in the study. The mean age of the BD group was 44.0 ± 12.4 years, and the mean age of the healthy control group was 42.8 ± 8.8 years. The BD and control groups were similar in terms of their age (p = 0.587) and sex (p = 0.764) distributions (Table 1).

Table 2. Basic characteristics of patients with BD (n: 49).

	Median (25th–75th quartiles)/n	%
Disease onset age	21.0 (17.0–27.0)	
First treatment age	22.0 (19.0–28.5)	
Number of hospitalizations	3.0 (3.0–6.0)	
Body mass index (kg/m^2)	31.1 (25.5–33.1)	
Consumes alcohol		
No	42	85.7%
Yes	7	14.3%
Smoker		
No	24	49.0%
Yes	25	51.0%
Marital status		
Not married	20	40.8%
Married	29	59.2%
Employment status		
Not working/retired	39	79.6%
Working	10	20.4%
Education status		
Primary-secondary school	19	38.8%
High school	21	42.9%
University	9	18.4%
Has a history of suicide attempt		
No	32	65.3%
Yes	17	34.7%
Has affective disorder in the family		
No	23	46.9%
Yes	26	53.1%
Has schizophrenia in the family		
No	43	87.8%
Yes	6	12.2%
Mood stabilizer used by the patient		
Valproic acid	26	53.1%
Lithium	10	20.4%
Combine	7	14.3%
None	6	12.2%
Receives antipsychotic treatment		
No	9	18.4%
Yes	40	81.6%

Values are presented as median (25th–75th quartiles) or frequency (%) for continuous and categorical variables, respectively.

Clinical Features of Participants in BD Group

In the BD group, the median age of onset of the disease was 21.0, and the median age at first treatment was 22.0. Mood stabilizers were used by 87.8% of the patients, while 81.6% were undergoing antipsychotic treatment (Table 2).

Peripheral Blood Levels of Molecules Associated with the Ubiquitination Process

The TDP-43 ($p < 0.001$), UCH-L1 ($p = 0.037$), UCH-L3 ($p = 0.007$), Histone Dea-1 ($p = 0.006$), Histone Dea-2 ($p = 0.047$), and ligase cullin-3 ($p = 0.031$) levels of the BD group were significantly lower than those of the control group. Cullin-3 levels did not differ significantly ($p = 0.188$) between the BD and control groups (Table 3).

Relationship between Blood Levels of Molecules and Sleep Quality

No significant ($p > 0.05$) correlation was observed between the TDP-43, UCH-L1, UCH-L3, Histone Dea-1, Histone Dea-2, cullin-3, and ligase cullin-3 levels of the patients and their PSQI, HAM-D, and YMRS scores (Table 4).

Table 3. Peripheral blood levels of examined molecules in the BD group (n: 49) and control group (n: 50).

	Healthy control group (n: 50)	BD group (n: 49)	*p*-value	*z*-value
	Median (25th–75th quartiles)	Median (25th–75th quartiles)		
TDP-43 (ng/mL)	1126.5 (756.6–2223.8)	805 (714–1634)	<0.001[m]	–3.555
UCH-L1 (ng/mL)	5.2 (3.5–9.1)	3.9 (3.6–6.9)	0.037 [m]	–2.082
UCH-L3 (ng/mL)	1.6 (1.0–3.0)	1.1 (1.0–2.2)	0.007 [m]	–2.708
Histone Dea-1 (ng/mL)	18.5 (13.1–33.0)	14.1 (13.1–24.8)	0.006 [m]	–2.743
Histone Dea-2 (ng/mL)	4.6 (3.6–8.5)	4 (3.6–6.0)	0.047 [m]	–1.984
Cullin-3 (ng/mL)	279.7 (199.2–573.8)	241.8 (218.2–434.5)	0.188 [m]	–1.316
Ligase cullin-3 (ng/mL)	412.7 (331.3–785.9)	354 (329–581)	0.031 [m]	–2.163

[m] Mann-Whitney U-test, values are presented as median (25th–75th quartiles). TDP-43, TAR DNA-binding protein-43; UCH-L1, ubiquitin C-terminal hydrolase-L1 enzyme; UCH-L3, ubiquitin C-terminal hydrolase-L3 enzyme; Histone Dea-1, histone deacetylase I; Histone Dea-2, histone deacetylase II.

Table 4. Correlations between peripheral blood levels of examined molecules and PSQI, HAM-D, and YMRS scores of BD group (n: 49).

		PSQI	HAM-D	YMRS
TDP-43	*r*	–0.015	0.070	0.000
	p	0.919	0.634	0.999
UCH-L1	*r*	0.095	0.148	–0.143
	p	0.517	0.309	0.328
UCH-L3	*r*	0.162	0.259	–0.104
	p	0.267	0.072	0.477
Histone Dea-1	*r*	0.028	0.073	–0.094
	p	0.849	0.617	0.522
Histone Dea-2	*r*	0.000	0.112	–0.100
	p	0.999	0.442	0.492
Cullin-3	*r*	0.115	0.167	–0.219
	p	0.430	0.253	0.130
Ligase cullin-3	*r*	0.122	0.110	–0.106
	p	0.403	0.453	0.468

Spearman Correlation

PSQI, Pittsburgh Sleep Quality Index; HAM-D, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

Discussion

The role of UPS, whose relationship we know better to circadian rhythm and sleep, in BD is not yet clear. In this study, we investigated the peripheral levels of molecules involved in the ubiquitination process and their relationship to sleep quality in individuals with BD. We found that the blood levels of TDP-43, UCH-L1, UCH-L3, Histone Dea-1, Histone Dea-2, and ligase cullin-3 were significantly lower in the BD group compared to the control group. However, we could not find a relationship between the blood levels of the examined molecules in the BD group and the sleep quality scores of the patients in the BD group.

There is evidence supporting the association of BD with many conditions such as inflammatory diseases, neuroplasticity disorder, oxidative stress, and mitochondrial disorder. Therefore, new biomarkers should be sought to identify and investigate associated biological processes. Brain-derived neurotrophic factor (BDNF) stands out as the most widely accepted biomarker in this context due to its contribution to the continuity of neuroplasticity, synaptic activity, and neurotransmitter regulation in studies conducted so far. The discovery of new and sensitive diagnostic markers in BD will lead to advances in the better understanding and treatment of the disease [25].

In the human body, all intracellular proteins undergo constant synthesis and degradation. In this continuous cycle, damaged or altered proteins are also eliminated. This continuous renewal of cellular components that have not yet lost their function affects cell viability positively by preventing the accumulation of damaged intracellular compounds [26]. Ubiquitin is a small polypeptide of 76 amino acids (8.5 kDa) that is highly conserved in all eukaryotic cells and is involved in protein degradation. Ubiquitin is conjugated to the protein to be degraded, and the protein with ubiquitin is selectively degraded by the proteasome, a multicatalytic enzymatic complex [5]. Besides the lysosomal system, UPS is one of the two main proteolytic systems responsible for the intracellular protein cycle [6]. UPS is the most important cellular mechanism in the destruction of misfolded, oxidized, and/or damaged abnormal proteins [27]. Moreover, what makes this system indispensable for cell viability is its function of recognizing protein degradation and its regulatory role in numerous intracellular processes. UPS plays a key role in the cell cycle, signal transduction, transcriptional regulation, DNA repair, stress response, programmed cell death, and antigen presentation, which are essential for cellular homeostasis. Therefore, it is thought that a disorder in one or more of the components of UPS may be among the important causes of diseases [7]. A possible loss of function in this pathway causes accumulation in target proteins, and an increase in function causes abnormal destruction in target proteins [28].

Dysfunction in UPS components has been reported to be associated with many neurological and mental diseases. UCH-L1, which functions as a deubiquitinating enzyme in UPS, takes part in the pathogenesis of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, spinocerebellar ataxia, and Huntington's disease. *UCH-L1* gene deletion has also been found to cause progressive loss of dopaminergic neurons in the substantia nigra and striatum. UCH-L1 is required for the maintenance of axonal integrity. In mice with UCH-L1 deficit, synaptic activity was also impaired. UCH-L1 deficit causes the accumulation of intracellular abnormal proteins and neurodegeneration [26,29]. It is thought that UCH-L1 is a neuronal antioxidant, and maintaining its normal levels is important for the protection of brain functions [12]. Ubiquitin ligase, another protein associated with ubiquitination, is crucial in substrate selection and functions in maintaining synaptic plasticity and regulating neurotransmitter receptors [30–32]. It was reported that BDNF expression is mediated by a ubiquitin ligase substrate, and chronic stress exposure causes a decrease in BDNF levels this way [33]. Changes in BDNF expression in major depressive disorder (MDD) are well known, but this is a new perspective. Recent studies have shown that ubiquitin ligase may be involved in the pathophysiology of BD [34,35]. Genes which encode ubiquitin ligase are negatively expressed in the frontal cortex of BD subjects [35]. Another molecule related to ubiquitination, TDP-43, has been thought to be associated with neurodegenerative diseases such as Amyotrophic lateral sclerosis and frontotemporal lobar degeneration [36]. It was reported that serum levels of TDP-43 and UCH-L1 are lower in autistic individuals, and abnormal ubiquitin ligase activity contributes to the neuropathological features of autism [37]. It is known that UPS plays an active role in sleep and circadian rhythm regulation [14]. The circadian system is a biological process adapted to the 24-hour cycle. This system regulates processes such as body temperature, nutritional activity, hormone release, and sleep-wake rhythm. Therefore, the circadian rhythm is an innate system that ensures the continuity of processes involving many functions related to survival and health [38]. It is the main factor in maintaining the sleep-wake cycle. Because of this function, it plays a key role in maintaining mood stability in BD [15]. Changes in the duration and quality of sleep are frequently observed in both manic and depressive episodes of BD. In Diagnostic and Statistical Manual of Mental Disorders, insomnia or hypersomnia for a depressive episode and a decrease in the need for sleep for a manic episode were counted among the basic diagnostic criteria [39]. In addition to being an important indicator of BD episodes, sleep disorders are observed even in the euthymic periods of patients [40]. Moreover, it was argued that sleep disorders may be a predisposing factor for BD [41]. It has been suggested that in the long term, sleep disorders, both as a factor and as a symptom, may play a decisive role in relation to clinical findings and the course of the disease. Poor sleep quality in individuals with BD is associated with a poor prognosis of the disease, decreased functionality, and lower quality of life [38,42]. The fact that sleep disorders are seen in all subtypes and stages of BD and affect the course of the disease indicates the importance of sleep and circadian rhythm in maintaining mood stability in BD [38].

The widely accepted cause of sleep disorders in BD is circadian system disorders. Many studies have shown that circadian rhythm disorders are detected in patients with BD, independent of mood episodes [16–18]. For example, it was reported that euthymic BD patients had lower melatonin secretion at night compared to healthy controls, and their melatonin peak time was delayed. In another study, it was shown that cortisol secretion was higher in depressive and hypomanic BD patients compared to healthy controls [16–18]. A study even defined the irregular circadian system as a biomarker for BD [43]. Additionally, it was stated that BD pharmacotherapy can positively affect abnormal circadian rhythm, and lithium is effective in regulating abnormal circadian cycle length [38].

It is not yet clear what role UPS, which we know is closely related to sleep and circadian rhythm regulation, plays in BD. In gene expression analysis studies, it was determined that patients with BD and schizophrenia had low expression levels in genes related to UPS [44,45]. In a study on whether single gene expressions could contribute to the pathogenesis of mental disorders, it was found that ubiquitin ligase and cullin protein encoded by the ubiquitin protein ligase E3A (*UBE3A*) gene were at low levels in BD patients. It was even stated that UBE3A could offer a potential biomarker function for BD with the help of bioinformatics technology to be integrated [35]. In another study, which included patients with schizophrenia and BD and investigated the relationship between positive psychotic symptoms and *UPS* gene expressions, a positive correlation was found between positive symptoms and ubiquitin conjugation gene expression, and a negative correlation was found between positive symptoms and deubiquitinating gene expression [46].

Several risk factors associated with the development of BD may be related to changes in different cellular pathways such as neuroplasticity [47], inflammation [48], oxidative stress [49], and cell death [50]. The oxygen consumption rate of the brain is high, and oxidative stress is more likely to occur in the brain than in other organs [51]. Oxidative stress is one of the factors responsible for protein malformation, and it was reported that there may be a dysfunction in the protein control mechanism in mood disorders [52]. Neurodegenerative diseases (NDD) are caused by the accumulation of misfolded proteins in certain areas of the brain [53]. Cognitive symptoms in diseases are often accompanied by psychiatric symptoms [54]. A recent study showed that mood disorders increase the risk of developing NDD later in life [55]. It has been reported that

common molecular mechanisms underlie mood disorders [54]. UPS plays a critical role in maintaining cellular homeostasis, with numerous studies linking it to mood disorders [56,57].

In this study, we investigated the relationship of molecules associated with the ubiquitination process to BD and sleep quality. Among the molecules whose peripheral levels we examined, we found that the peripheral levels of TDP-43, UCH-L1, UCH-L3, ubiquitin ligase, Histone Dea-1, and Histone Dea-2 were significantly lower in the BD group than in the control group. However, we did not find a relationship between the peripheral blood levels of these molecules and the sleep quality scores in the BD group. The BD group included individuals who were continuing their follow-up at the community mental health center regularly, whose disease was in remission, and almost all of them were using mood stabilizers or antipsychotic drugs. Many of these drugs have a hypno-sedative effect, and this may have obscured the relationship between sleep quality and ubiquitination-related molecules. In other words, some patients may have good sleep quality, but their circadian rhythms may still have been disrupted.

The most important limitation of this study was that the effects of drugs, especially mood stabilizers and antipsychotics used by the patient group, on the peripheral blood levels of the investigated molecules could not be examined. Therefore, in future studies, the medication effects of mood stabilizers and antipsychotics should be taken into account, or a medication-free subgroup should be included to better isolate the impact of BD itself on the ubiquitination process. Additionally, there is a need for larger samples. Although the numbers of individuals in the BD and healthy control groups were enough according to the power analysis that was conducted in this study, expanding the sample size in future studies would allow the examination of other possible factors such as age, sex, the age of onset of the disease, the number of episodes, family history, the presence of psychotic symptoms, and other parameters.

Conclusions

The data we obtained are important to understand the relationship between BD and the ubiquitination process. These data both support our literature-based knowledge and show that the ubiquitination process can be affected in BD patients without being associated with sleep quality. Further studies in the field are needed to understand whether this interaction is mediated by the circadian system, which has an active role in BD, or whether different pathways are active.

Abbreviations

BD, bipolar disorder; UPS, ubiquitin-proteasome system; TDP-43, TAR DNA-binding protein-43; UCH-L1, ubiquitin C-terminal hydrolase-L1 enzyme; UCH-L3, ubiquitin C-terminal hydrolase-L3 enzyme; Histone Dea-1, histone deacetylase I; Histone Dea-2, histone deacetylase II; YMRS, Young Mania Rating Scale; HAM-D, Hamilton Depression Rating Scale; PSQI, Pittsburgh Sleep Quality Index.

Availability of Data and Materials

The datasets used or analyzed during the present study are available from the corresponding author upon reasonable request.

Author Contributions

ÜA, EY, İÇ, and HK designed the study. ÜA, EY, İÇ, and HK performed the research. HK analyzed the data. ÜA and EY wrote the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The experimental protocol was established according to all applicable ethical guidelines and recommendations of the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (B.30.2.ODM.0.20.08/112-270). Forty-nine participants diagnosed with BD and 50 healthy volunteers of similar age and sex distributions whose written informed consent was obtained were included in the study.

Acknowledgment

Not applicable.

Funding

This work was supported by the “Hitit University” as a Scientific Research Project [Project Code: TIP19001.19.005].

Conflict of Interest

The authors declare no conflict of interest.

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