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High Mobility Group Box 1 Levels as an Inflammatory Mediator in Bipolar Mania

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ABSTRACT

Introduction: High mobility group box 1 protein (HMGB1) is a member of the molecular family known as damage-associated molecular patterns, which is implicated to have a role in neuroinflammation processes. In recent years, a growing number of studies have focused on the role of inflammation in Bipolar Disorder (BD). This study aimed to investigate the serum levels of HMGB1 and other inflammatory markers in patients with bipolar manic episodes compared to those in healthy controls (HC).

Methods: A single-center, observational, case-control study was conducted. Thirty-five patients with BD in manic episodes and 35 HC were assessed. Young Mania Rating Scale (YMRS) was used to assess the symptom severity of the patient group. While inflammatory markers (such as HMGB1, C-reactive protein (CRP) and white blood cell count) were assessed at the first three and the last day of hospitalization in the patient group, they were evaluated once in HC. Levels of inflammatory markers were compared between (patient-HC) and within groups (before-after treatment).

Results: No difference was observed in serum HMGB1 levels of bipolar patients with manic episodes compared to the HC ($p>0.05$). C-reactive protein levels of manic patients were higher than HC ($p<0.001$), and the difference persisted even after treatment ($p=0.007$). In addition, there was a significant positive correlation between CRP levels and antipsychotic drug dosage ($r=0.382$, $p=0.024$).

Conclusion: There were no differences in HMGB1 levels between bipolar patients with acute manic episode and HC. However, higher CRP levels in bipolar patients support the low-grade inflammation hypothesis in the etiology of BD.

Keywords: Bipolar disorder, CRP, HMGB1, Inflammation, Mania

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INTRODUCTION

Bipolar disorder (BD) is a severe psychiatric disorder that affects 2.5% of the population and is usually progressive with periods of recurrent mood exacerbations (1). Chronic medical conditions, especially immunological disorders such as autoimmune thyroiditis, systemic lupus erythematosus and multiple sclerosis are more prevalent among patients with BD than in the general populations (2). Bipolar disorder and accompanying medical conditions decrease the quality of life and lead to functional impairment, which makes BD one of the most disabling medical conditions (3). A hypothesis proposed in 1980 stated that immune activity exacerbations could trigger attacks in BD, as observed in autoimmune diseases. Moreover, lithium has been suggested as a potential immune modulator (4). Since then, several researchers have investigated the interaction between BD and immune dysfunction. Substantial evidence has demonstrated immunological alterations in BD during the past decades. Therefore, it is possible to consider BD as a multisystem condition rather than just a brain disorder (3). Despite the growing evidence for the inflammation hypothesis, a specific inflammatory marker has still not been found. Neutrophils, lymphocyte counts, ratios, cytokines, and C-reactive protein (CRP) are the most researched markers for the inflammation hypothesis. Meta-analyses have consistently shown increased CRP levels in BD (5). However, the relationship between CRP levels, symptom severity, age, and disease duration remains unclear.

Highlights

- **Higher CRP levels in patients support the low-grade inflammation hypothesis in bipolar disorder (BD).**
- **HMGB1 is not considered a good indicator of acute manic episodes of BD.**
- **There is a positive correlation between CRP levels and daily doses of antipsychotic drugs.**
- **NLR and PLR values increased during the treatment of mania.**

High mobility group box 1 protein (HMGB1) is a non-histone protein showing high electrophoresis mobility (6). It functions as a DNA chaperone that regulates chromatin structure, gene expression and DNA repair in the cell nucleus. At the same time, it acts as a proinflammatory molecule that is released extracellularly in case of damage and initiates inflammatory events (7). It is a member of the molecular family known as damage-associated molecular patterns (DAMP), which stimulates the immune system, initiates the inflammatory response, and triggers the

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regeneration process (8). Positive correlation between levels of HGMB1 and CRP was shown in some pathologies (9). In addition, HMGB1 is defined as a more sensitive biomarker than CRP (10). HMGB1 levels increased in many autoimmune and inflammatory diseases, such as chronic rheumatic diseases, diabetes, coronary syndrome, brain damage (11–13). In addition, it is implicated to have a role in neuroinflammation and neurodegeneration processes, such as in the memory deficits observed in Parkinson's Disease and Alzheimer's Disease (13–15). It has been implied that the persistent cognitive damage seen in people recovering from sepsis may be related to increased levels of HMGB1 (16).

In recent years, HMGB1 levels have been investigated in psychiatric disorders. However, studies in this area are relatively new and very limited. Emanuel et al. showed that the HMGB1 level was higher in autism spectrum disorders than in healthy controls (HC) (17). In 2015, Wu et al. examined the role of HMGB1 in the development of depression (18). They concluded that HMGB1 is secreted actively in the central nervous system (CNS), and HMGB1 has a role in depression triggered by lipopolysaccharide (18). Few studies have shown increased HMGB1 levels in patients with alcohol addiction (19), autism (12,17), anorexia nervosa (20), major depression (21,22) and schizophrenia (23–25). A pilot study examined the plasma level of HMGB1 in euthymic patients with BD, and HMGB1 levels were found to be higher in euthymic patients with BD than in healthy controls (HC) (10). However, we have not found any study investigating the level of HMGB1 in patients with BD manic episodes. Thus, for the first time, we aimed to investigate the HMGB1 levels in patients with acute manic episodes during hospitalization and compare them with the HC. We hypothesized that i) Serum HMGB1, CRP, neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) levels are relatively higher in patients compared to HC, ii) Serum HMGB1 and CRP levels of patients decrease after treatment compared to pre-treatment, iii) There is a positive correlation between serum HMGB1 and CRP levels, iv) There is a positive correlation between disease severity and serum HMGB1 and CRP levels.

METHODS

A single-center, observational, case-control study was designed.

Bakırköy Dr. Sadi Konuk Training and Research Hospital's Clinical Ethics Committee approved the study with the protocol code 2020/490 in 16.11.2020. A written informed consent was obtained from all subjects who participated in the study.

Subject

Data were collected from the patients with 'Bipolar Disorder Manic Episodes' according to the criteria of the Diagnostic Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Patients were screened from November 2020 to April 2021. Seventy-five patients with BD and thirty-five HC were interviewed. Patients were interviewed twice (one of the first three days of hospitalization and the day which they were discharged). All patients and HC were between 18 and 65 years of age. Patients were selected regardless of their psychotropic medication status or previous number of episodes. The exclusion criteria were as follows: i) having a diagnosis of neurological disease, inflammatory disease, autoimmune disease, diabetes mellitus, chronic heart disease and a history of head trauma; ii) having a diagnosis of alcohol or substance use disorder; iii) history of operation or myocardial infarction in the last six months, iv) being pregnant or breastfeeding, v) using steroids, immunomodulators and similar drugs that affect the immune system, vi) detection of >37°C measurements in body temperature follow-ups during the study.

Assessment

Each patient and HC underwent a baseline assessment, including a clinical interview conducted by a psychiatrist to confirm the diagnoses of BD with manic episode. Young-Mania Rating Scale (YMRS) (26,27) and Hamilton Depression Scale (HAM-D) (28,29) were used to measure symptom severity. The baseline assessment included the sociodemographic data and anthropometric measurements (Body mass index (BMI) and waist circumferences). For the patient group, a follow-up clinical assessment was conducted at the end of hospitalization. At the baseline and follow-up assessments, blood samples were collected from each patient for serum HMGB1 and CRP levels and the white blood cell (WBC) count. The same sociodemographic data, anthropometric measurements, and blood samples were collected only at baseline in the HC group.

Measurement of Biochemical Data

Serum CRP levels and WBC counts were recorded as baseline levels from routine blood samples taken in the first three days of hospitalization. In order to study serum HMGB1 levels, peripheral venous blood samples collected in tubes with a capacity of 5 ml and containing ethylene diamine tetraacetic acid (EDTA) were kept at room temperature for two hours or at +4°C overnight, then centrifuged at 1000 x g for 20 minutes and stored at -80°C until the serum samples were studied in the biochemistry laboratory. Concentrations of HMGB1 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Human HMGB1 ELISA kit by FineTest, Wuhan). The same procedures were repeated on the day the patient was discharged. Since HMGB1 has no circadian rhythm (10,30), no fixed time interval was determined for blood sample collection.

Statistical Analysis

Power analysis was performed by G*Power (University of Dusseldorf). To achieve a power of 0.95 with a type 1 error of 5%, the minimum sample size for each group (patient and control groups) was 29 with an expected large effect size. The expected effect size was calculated from the data of a previously published study that investigated HMGB1 plasma levels in patients with BD (13). Considering the high probability of dropout rate in patients with BD, we decided to recruit more than 29 subjects for the patient group.

The normality of continuous variables was evaluated by Shapiro-Wilk test and Q-Q normality curves. Data were presented as mean ± standard deviation or median (first to the third quartile) based on the distribution patterns. Normally distributed data were compared with independent samples t-test for between-group comparison and paired samples t-test for within-group comparison. Mann-Whitney U test (between-group) and Wilcoxon signed-rank test (within-group) were used for non-normally distributed data. Categoric variables were presented as numbers and percentage. Chi-square test was used for between-group comparisons. However, Fisher's exact test was performed when more than 25% of cells had a count lower than the expected 5. Spearman correlation analysis was used to evaluate the correlation between two continuous variables or between a continuous variable and a binary categorical variable. Finally, multiple regression analysis was run to investigate the factors (age, gender, BMI and smoking status) affecting HMGB1 and CRP levels together. IBM Statistical Package for Social Sciences (SPSS) program version 26.0 (Armonk, NY: IBM Corp.) was used to analyze the data. The significance level for all analyses was determined as $p<0.05$.

RESULTS

Sociodemographic and Clinical Characteristics

Seventy-five patients with BD were interviewed, but forty patients were excluded from the study due to clinical and laboratory findings of inflammation during the follow-up period or early discharge from hospital before the completion of treatment. We completed the study with 35 patients and 35 HC. Eighteen (51%) of the patients and healthy controls were female, and 17 (49%) were male. The mean age of the

Table 1. Clinical features for patients group

Age of onset (year)#	24 (18–30.5)
Age of first treatment (year)#	26 (19.5–31.5)
Duration of illness (year)#	8 (4.5–18)
Number of hospitalizations#	3 (1–5)
Number of manic episodes#	3 (2–5)
Number of depressive episodes#	1 (0–2)
First episode of the disease	
Mania	20 (57%)
Depression	15 (43%)
Mixed features	
present	12 (34%)
absent	23 (66%)
Seasonality	
present	9 (26%)
absent	26 (74%)
Medication compliance	
present	13 (37%)
absent	22 (63%)
Suicide attempt	
present	8 (23%)
absent	27 (77%)
Psychotropic medication use before hospitalization	
present	5 (14%)
absent	30 (86%)
Psychotic features	
present	32 (91%)
absent	3 (9%)
ECT	
present	3 (9%)
absent	32 (91%)
Antipsychotic drug during the treatment	
Atypical	26 (74%)
Typical	4 (11%)
Atypical+Typical	13 (37%)
Antipsychotic drug doses 1=100 mg/day KLP+	9.91 (±2.43)
Mood Stabilizer during the treatment	
Li	20 (57%)
VPA	11 (31%)
CMZ	1 (3%)
Li+VPA	1 (3%)
Li+LMJ	2 (6%)
Days of hospitalization (day)#	17 (13–22)

Data are presented #as median (first-third quartile) and +mean (± standard deviation).
Other data are presented as numbers (%).
CMZ: carbamazepine; ECT: electroconvulsive therapy, KLP: chlorpromazine; Li: lithium; LMJ: lamotrigine; VPA: valproic acid.

patients was 37.54±11.59 (minimum: 18, maximum: 60), and 36.54±10.12 (minimum: 21, maximum: 58) in the control group (p>0.05). The median amount of smoking, 1 (0–1.5) pack/day for the patient group, was significantly higher compared to the control group, which was 0 (0–1) pack/day (p=0.002).

Comparison of psychometric scales between baseline and the discharge day among the patient group showed a statistically significant (p<0.001) reduction from 44 (37–49) to 2 (0–4) in manic symptoms severity.

The clinical characteristics of the patients were summarized in Table 1.

Laboratory Results Screening

We observed significantly higher CRP levels in patients with BD. Even though CRP levels decreased after treatment, significant differences still existed. Additionally, the rates of PLR and NLR increased significantly after the treatment. However, no significant difference was found in HMGB1 values between patients and HC. Also, there was no significant change in HMGB1 values with treatment in the short term. Pairwise comparisons between the HC and patients (Table 2) and patients before and after treatment (Table 3) were shown. In addition, the comparison between HC and patients after treatment was demonstrated in Table 4.

There was no significant correlation between HMGB1 level and the total number of manic episodes, total disease duration, and age at the onset of the disease (p>0.05). However, there was a significant positive correlation between CRP and the antipsychotic drug dose (r=0.382, p=0.024). In addition, there was also a positive correlation between antipsychotic

Table 2. Comparison of laboratory screening and anthropometric measurements between patient and healthy controls

	Patient Group (n=35)	HC Group (n=35)	p-value
Anthropometric Values			
BMI (± sd)⁺	**29.43 (±6.74)**	**25.24 (±3.90)**	**0.002****
Waist circumference (cm)(min-max)#	**94 (85–105)**	**81 (71.5–95.5)**	**0.011***
Laboratory values			
HMGB1 (pg/ml) (min-max)#	52.96 (49.27–60.2)	53.25 (49.19–59.2)	0.856
CRP (mg/L) (min-max)#	**3.78 (1.68–6.6)**	**1.06 (0.55–2.36)**	**<0.001****
WBC (min-max)#	8.42 (7.01–9.41)	8.15 (6.66–8.97)	0.267
NE (min-max)#	4.59 (4.14–6.1)	4.84 (3.81–5.78)	0.526
PLT (± sd)⁺	258.09 (±54.45)	264.49 (±51.91)	0.616
NLR (min-max)#	1.9 (1.62–2.3)	2.07 (1.62–2.26)	0.948
PLR (min-max)#	101.95 (87.27–117.18)	109.83 (94–131.73)	0.238
MLR (± sd)⁺	0.22 (±0.07)	0.20 (±0.07)	0.18
PMI (± sd)⁺	2622.67 (479.36)	2715.66 (±434.96)	0.398
MPV (± sd)⁺	10.27 (±1.06)	10.39 (±1.10)	0.636

⁺Independent t test was used and data were presented as mean (± standard deviation).
#Mann-Whitney U test was used and data were presented as median (first-third quartile).
*statistically significant with p<0.05 , **statistically significant with p<0.005.
BMI: body mass index; CRP: C-reactive protein; HMGB1: high mobility group box-1 protein; max: maximum; min: minimum; MLR: monocyte-to-lymphocyte ratio; MPV: mean platelet volume; NE: neutrophil count, NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; PLT: platelet count; PMI: platelet mass index; sd: standard deviation WBC: white blood cell.

Table 3. Comparison of YMRS and laboratory screening of patients before and after treatment

	Before treatment (n=35)	After treatment (n=35)	p-value
Psychometric Scale			
YMRS (min-max)#	**44 (37–49)**	**2 (0–4)**	**<0.001****
Laboratory Values			
HMGB1 (pg/ml)(min-max)	52.96 (49.27–60.2)	54.44 (49.4–59.33)	0.987
CRP (mg/L)(min-max)#	3.78 (1.68–6.6)	2.17 (0.1–5.17)	0.262
WBC (min-max)#	8.42 (7.01–9.41)	8.85 (7.72–10.1)	0.199
NE (min-max)#	4.59 (4.14–6.1)	5.82 (4.34–6.44)	0.05
PLT (min-max)#	255 (215–290)	248 (215–278)	0.7
NLR (min-max)#	**1.9 (1.62–2.3)**	**2.2 (1.93–3.99)**	**0.003****
PLR (± sd)⁺	**103.75 (±26.99)**	**118.1 (±35.24)**	**0.026***
MLR (min-max)#	0.21 (0.18–0.25)	0.19 (0.17–0.27)	0.213
PMI (min-max)#	2638.4 (2341.1–2885)	2502.9 (2199.8–2786.75)	0.295
MPV (± sd)⁺	10.27 (±1.06)	10.2 (±1.07)	0.603

#Wilcoxon signed-rank test was used and data were presented as median (first-third quartile).
⁺paired samples t-test was used and data were presented as mean (± standard deviation).
*statistically significant with p<0.05, **statistically significant with p<0.005.
BMI: body mass index; CRP: C-reactive protein; HMGB1: high mobility group box-1 protein; max: maximum; min: minimum;MLR: monocyte-to-lymphocyte ratio; MPV: mean platelet volume; NE: neutrophil count, NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; PLT: platelet count; PMI: platelet mass index; sd: standard deviation WBC: white blood cell; YMRS: Young Mania Rating Scale.

Table 4. Comparison of laboratory screening between healthy controls and patients after treatment

	After Treatment (n=35)	Healthy Controls (n=35)	p-value
Laboratory Values			
HMGB1 (pg/ml)	54.44 (49.4–59.33)	53.25 (49.19–59.20)	0.986
CRP (mg/L)	**2.17 (0.1–5.17)**	**1.06 (0.55–2.36)**	**0.007***
WBC	8.85 (7.72–10.1)	8.15 (6.66–8.97)	0.61
NE	**5.82 (4.34–6.44)**	**4.84 (3.81–5.79)**	**0.019***
PLT	248 (215–278)	255 (228.5–293)	0.375
NLR	**2.2 (1.93–3.99)**	**2.07 (1.62–2.26)**	**0.029***
PLR	116.49 (92.39–141.04)	109.83 (94–131.73)	0.459
MLR	0.19 (0.17–0.27)	0.2 (0.15–0.22)	0.267
PMI	2502.9 (2199.8–2786.75)	2601 (2393.8–3043.5)	0.122
MPV⁺	10.2±1.07	10.39±1.10	0.465

⁺Independent t test was used and data were presented as mean (± standard deviation). Mann-Whitney U test was used for other variables and data were presented as median (first-third quartile).
*statistically significant with p<0.05.
CRP: C-reactive protein; HMGB1: high mobility group box-1 protein; MLR: monocyte-to-lymphocyte ratio; MPV: mean platelet volüme; NE: neutrophil count, NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; PLT: platelet count; PMI: platelet mass index; WBC: white blood cell.

Table 5. Investigation of factors affecting HMGB1 level by multiple regression analysis

HMGB1 level	B	95% CI for B		SE B	Beta	R^2	Adjusted R^2	p
		LL	UL					
Model						0.29	0.24	**<0.001***
Constant	92.43	72.03	112.84	10.21				**<0.001***
Group	-2.52	-8.45	3.41	2.97	-0.1			0.4
Age	-0.05	-0.31	0.21	0.13	-0.04			0.71
Sex	-8.73	-14	-3.44	2.64	-0.36			**0.002***
BMI	-0.55	-1.06	-0.03	0.26	-0.26			**0.038***
Smoking	-3.55	-6.63	-0.44	1.56	-0.27			**0.026***

Model: SPPS is the "Enter" method in statistics.
B: unstandardized regression coefficient; Beta: standardized coefficient; BMI: body mass index; CI: confidence interval; HMGB1: high activity group box 1 protein; LL: lower limit; R^2: determination variable; UL: upper limit; SE: standard error.
*p<0.05 denotes follow-up observation.

Table 6. Investigation of factors affecting CRP level by multiple regression analysis

CRP level	B	95% CI for B		SE B	Beta	R^2	Adjusted R^2	p
		LL	UL					
Model						0.3	0.24	**<0.001***
Constant	2.06	-2.8	6.91	2.43				0.4
Group	-2.45	-3.87	-1.04	0.7	-0.42			**0.001***
Age	0.03	-0.03	0.09	0.03	0.11			0.36
Sex	0.66	-0.6	1.91	0.63	0.11			0.3
BMI	0.11	-0.01	0.23	0.06	0.22			0.08
Smoking	-0.65	-1.39	0.09	0.37	-0.2			0.08

Model: SPPS is the "Enter" method in statistics.
B: unstandardized regression coefficient; Beta: standardized coefficient; BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; LL: lower limit; R2: Coefficient of determination; SE: standard error; UL: upper limit.
*p<0.05 indicates statistical significance.

drug dose and age of disease onset (r=0.390, p=0.02) and YMRS scale (r=0.471, p=0.004).

There was no correlation between HMGB1 levels and CRP, NLR, PLR and other inflammatory markers (p>0.05). However, we found positive correlations between CRP and leucocytes (r=0.487, p=0.003), neutrophils (r=0.463, p=0.005) and monocyte-lymphocyte ratio (MLR) (r=0.413, p=0.014). The multiple regression model, including disease presence, age, gender, BMI, and smoking, significantly predicted the HMGB1 level (F (5,64)=5.277, p<0.001, adjusted (adj) R^2=0.24). However, only gender (p=0.002), BMI (p=0.038), and smoking (p=0.026) significantly contributed to the regression model (Table 5).

The multiple regression model, including the presence of BD, age, gender, BMI, and smoking, significantly predicted the CRP level (F (5.64)=5.42, p<0.001, adj R^2=0.243). However, only the presence of BD significantly contributed to the regression model (Table 6).

DISCUSSION

To the best of our knowledge, this is the first study that screened for the serum HMGB1 in BD patients with manic episodes. Contrary to our hypothesis, there was no significant difference in serum HMGB1 levels of manic patients compared to HC. In addition, there was no significant difference in serum HMGB1 levels after the hospitalization period compared to the baseline. Consistent with previous studies, CRP levels of manic patients were significantly higher than HC, and the difference persisted even after treatment.

Measuring HMGB1 levels in acute episodes could be one of the reasons why no difference was found in HMGB1 levels of patients and HC. Because HMGB1 is one of the late-phase mediators of the inflammation process in contrast to TNF-α and IL-1, which are released during the earlier phases (18). Since this study assessed bipolar patients with manic episodes in the beginning and the last day of hospitalization (median duration was 17 days), even though YMRS of patients decreased considerably, we did not assume that these patients were in euthymic phases, considering that many studies use at least three months of remission as an inclusion criterion for assessing the euthymic period (1,10). Marie-Claire et al. reported that HMGB1 levels were higher in euthymic patients than in HC. However, contrary to our study, they found no differences in CRP levels (10). Considering HMGB1 as possibly a late mediator of inflammation, we can speculate that CRP may be a better indicator during acute episodes, whereas HMGB1 is a better indicator during the euthymic period. Additionally, HMGB1 levels were found to be higher in patients with schizophrenia than in HC during the remission phase (23). However, there was no specific change in HMGB1 levels in acute psychotic phases, interpreted as HMGB1 being a better indicator for the chronic remission period (23). In order to better interpret the effect of HMGB1, further studies are needed with BD patient groups longitudinally. In our study, although the YMRS decreased, the follow-up period was relatively short, i. e., 17 days (shown in Table 1). We speculate that the lack of change in HMGB1 levels may be related to the short follow-up period.

Kozlowska et al. reported that male patients' serum IL-33, sST2 and HMGB1 levels were significantly higher than HC, but no such difference was found in females. They also showed that CRP levels were higher in the patient's group, but there was no correlation between CRP and HMGB1 levels (25). We have not found any correlations between HMGB1 and other inflammatory markers such as CRP, NLR, and PLR. However, Marie-Claire et al. found a positive correlation between HMGB1 and CRP in euthymic bipolar patients (10).

There were significant differences between the groups regarding smoking status and BMI; therefore, we created a multiple regression model to investigate the effect of confounding factors such as age, gender, BMI, and smoking status on HMGB1 level. Finally, we found that gender, BMI, and smoking status affected HMGB1 levels, while having BD did not affect HMGB1 levels. In contrast to our study, gender, BMI and smoking status had no significant effect on HMGB1 levels in euthymic bipolar patients (10). The differences among the studies may be related to different methodological approaches. For example, the study investigating euthymic bipolar patients has a smaller sample size. In addition, they could not calculate the effect of psychotropic treatment. In their study, there were statistical differences between ages, but there was no difference in BMI status compared to ours. Moreover, the inclusion criteria were not defined in the previous bipolar study (10), which may have contributed to the significant differences.

Another finding of our study was higher CRP levels in patients than in HC. This difference existed even after reassessing the analysis with confounding factors such as gender, age, smoking status and BMI. It is well known that CRP is an important indicator of inflammation. A meta-analysis including twenty-seven studies showed that CRP levels were higher in every state of BD; however, the highest levels were observed in manic state compared to depression and euthymic state (5). They also stated that there was no relation between CRP levels and age, gender, smoking status, BMI and YMRS scores. In line with this, we also did not find any effect of age, gender, BMI and smoking status on CRP levels. However, other researchers have reported a relationship between CRP and YMRS scores (31). Even the slightest increase in CRP levels can be interpreted as low-grade inflammation (32). High peripheral CRP levels increase the permeability of the blood-brain barrier and allow it to pass into the CNS, thus directly affecting the brain (33). Some researchers consider that there is an inflammatory subgroup of BD which can be treated better with adjunctive anti-inflammatory medications (34). They also stated that combining anti-inflammatory treatments with mood stabilizers may reduce comorbidities and prevent disease progression (34).

We also analyzed the alterations in CRP levels in patients after short-term treatment. Even though the CRP levels were higher before treatment, we could not find significant changes in CRP levels after treatment. Both before and after treatment, CRP levels were significantly higher in patients with BD than in HC. Uyanik et al. prospectively investigated cytokines and hs-CRP (high sensitive CRP) levels before and after four weeks of treatment in BD patients and reported that levels decreased significantly after treatment (35). They also stated that YMRS scores were correlated with hs-CRP levels after treatment. Therefore, they suggested that hs-CRP was the strongest indicator among other inflammatory markers to determine the treatment response (35). In contrast, there was no correlation between YMRS and CRP levels in our study ($p>0.05$); however, the patients with higher CRP levels were observed to use higher doses of antipsychotic drugs daily during hospitalization period ($p=0.024$, $r=0.382$). C-reactive protein levels of patients decreased in time; however, the reason for insignificance in this reduction may be related to the short follow-up period.

There were no differences in NLR, PLR, MLR, leukocyte and neutrophil levels between HC and patients before treatment. Nevertheless, the neutrophil counts and NLR values were lower in HC than the ones in patients after treatment. We also found a significant increase in patients' NLR and PLR values after treatment. Munkholm et al. reported that neutrophil and leukocyte levels were higher in all episodes of BD patients without significant differences (36). Moreover, they also suggested that the levels of neutrophils and leukocytes in BD positively correlate with lithium use (36). Fusar-Poli et al. found that PLR, NLR, and MLR levels were higher during manic episode than in depressive episode, but they suggested that only the PLR value was a determiner for manic episode after the multiple regression analysis (37). While lymphocytes play a more significant role in the adaptive immune system, neutrophils are the first-line defense of the innate immune system (38). Thus, the increase in NLR values may reflect the activation of innate immunity.

This study has several strengths and limitations. Firstly, all patients had severe symptoms which required hospitalization, therefore the patients can not reflect the general mania population. In addition, since the patients were treated with high-dose and different kinds of psychotropic medication, chlorpromazine equivalent doses were used to analyze data. Secondly, some patients were using psychotropic medications before the blood samples were taken, which caused difficulty in interpreting some results. Thirdly, potential confounding factors on inflammatory statuses, such as sleep, exercise and nutrition, could not be included in the analysis. However, smoking status, BMI and waist circumference were included in the analysis. There were no differences among groups in terms of age and gender; all of these can be considered a strength of this study. We followed patients during hospitalization to analyze the short-term results of treatments on inflammatory markers but were unable to analyze the results of different mood episodes that could be detected in follow-up studies. Finally, we excluded the neurological, immune-inflammatory, and chronic heart disease cases, which may be an advantage for evaluating inflammatory markers in BD. However, it may lead to the exclusion of the "inflammatory subgroup of patients with BD", which could be considered a study limitation.

The most prominent finding of this study was the absence of significant differences in HMGB1 levels between patients and healthy groups, which might be due to HMGB1 being one of the late mediators of inflammation and having no immediate rise during acute episodes. We found that CRP levels were higher in manic patients, which supported the low-grade inflammation hypothesis in the etiology of BD. However, the inflammatory process involves several complex mechanisms, which makes it difficult to conclude the study without the longitudinal follow-up of the patients. Further longitudinal studies with larger samples and patient groups representing the entire BD universe are needed to better understand BD's etiopathogenesis and improve treatment options and diagnostic laboratory findings.

Ethics Committee Approval: Bakırköy Dr.Sadi Konuk Training and Research Hospital's Clinical Ethics Committee approved the study with the protocol code 2020/490 in 16.11.2020.

Informed Consent: A written informed consent was obtained from all subjects who participated in the study.

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REFERENCES

1. Melo MCA, Garcia RF, de Araújo CFC, Abreu RLC, de Bruin PFC, de Bruin VMS. Clinical significance of neutrophil-lymphocyte and platelet-lymphocyte ratios in bipolar patients: an 18-month prospective study. Psychiatry Res. 2019;271:8–14. [Crossref]
2. Rosenblat JD, McIntyre RS. Bipolar disorder and immune dysfunction: epidemiological findings, proposed pathophysiology and clinical implications. Brain Sci. 2017;7(11):3–5. [Crossref]
3. Fries GR, Walss-Bass C, Bauer ME, Teixeira AL. Revisiting inflammation in bipolar disorder. Pharmacol Biochem Behav. 2019;177:12–19. [Crossref]

4. Rosenblat JD. Targeting the immune system in the treatment of bipolar disorder. Psychopharmacology (Berl). 2019;236(10):2909–2921. [Crossref]
5. Fernandes BS, Steiner J, Molendijk ML, Dodd S, Nardin P, Gonçalves CA, et al. C-reactive protein concentrations across the mood spectrum in bipolar disorder: a systematic review and meta-analysis. Lancet Psychiatry. 2016;3(12):1147–1156. [Crossref]
6. Ueda T, Yoshida M. HMGB proteins and transcriptional regulation. Biochim Biophys Acta Gene Regul Mech. 2010;1799(1-2):114–118. [Crossref]
7. Li J, Kokkola R, Tabibzadeh S, Yang R, Ochani M, Qiang X, et al. Structural basis for the proinflammatory cytokine activity of high mobility group box 1. Mol Med. 2003;9(1-2):37–45. [Crossref]
8. Takizawa T, Shibata M, Kayama Y, Shimizu T, Toriumi H, Ebine T, et al. High-mobility group box 1 is an important mediator of microglial activation induced by cortical spreading depression. J Cereb Blood Flow Metab. 2017;37(3):890–901. [Crossref]
9. Ciucci A, Gabriele I, Percario ZA, Affabris E, Colizzi V, Mancino G. HMGB1 and cord blood: Its role as immuno-adjuvant factor in innate immunity. PLoS One. 2011;6(8):1–10. [Crossref]
10. Marie-Claire C, Courtin C, Curis E, Bouaziz-Amar E, Laplanche JL, Jacob A, et al. Increased plasma levels of high mobility group box 1 protein in patients with bipolar disorder: a pilot study. J Neuroimmunol. 2019;334:576993. [Crossref]
11. Richard SA, Min W, Su Z, Xu H-X. Epochal neuroinflammatory role of high mobility group box 1 in central nervous system diseases. AIMS Mol Sci. 2017;4(2):185–218. [Crossref]
12. Dipasquale V, Cutrupi MC, Colavita L, Manti S, Cuppari C, Salpietro C. Neuroinflammation in autism spectrum disorders: Role of high mobility group box 1 protein. Int J Mol Cell Med. 2017;6(3):148–155.
13. Baran A, Bulut M, Kaya MC, Demirpençe Ö, Sevim B, Akıl E, et al. High-sensitivity C-reactive protein and high mobility group box-1 levels in Parkinson's disease. Neurol Sci. 2019;40(1):167–173. [Crossref]
14. Fang P, Schachner M, Shen YQ. HMGB1 in development and diseases of the central nervous system. Mol Neurobiol. 2012;45(3):499–506. [Crossref]
15. Festoff BW, Sajja RK, van Dreden P, Cucullo L. HMGB1 and thrombin mediate the blood-brain barrier dysfunction acting as biomarkers of neuroinflammation and progression to neurodegeneration in Alzheimer's disease. J Neuroinflammation. 2016;13(1):194. [Crossref]
16. Chavan SS, Huerta PT, Robbiati S, Valdes-Ferrer SI, Ochani M, Dancho M, Frankfurt M, Volpe BT, Tracey KJ, Diamond B. HMGB1 mediates cognitive impairment in sepsis survivors. Mol Med. 2012 Sep 7;18(1):930-7. [Crossref]
17. Emanuele E, Boso M, Brondino N, Pietra S, Barale F, Ucelli di Nemi S, et al. Increased serum levels of high mobility group box 1 protein in patients with autistic disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(4):681–683. [Crossref]
18. Wu TY, Liu L, Zhang W, Zhang Y, Liu YZ, Shen XL, et al. High-mobility group box-1 was released actively and involved in LPS induced depressive-like behavior. J Psychiatr Res. 2015;64:99–106. [Crossref]
19. Whitman BA, Knapp DJ, Werner DF, Crews FT, Breese GR. The Cytokine mRNA Increase induced by withdrawal from chronic ethanol in the sterile environment of brain is mediated by CRF and HMGB1 release. Alcohol Clin Exp Res. 2013;37(12):2086–2097. [Crossref]
20. Yasuhara D, Hashiguchi T, Kawahara K, Nakahara T, Harada T, Taguchi H, et al. High mobility group box 1 and refeeding-resistance in anorexia nervosa. Mol Psychiatry. 2007;12(11):976–977. [Crossref]
21. Demir S, Bulut M, Kaya M, Sevim B, Demirpence O, Ibiloglu A, et al. Increased high mobility group box 1(HMGB1) level in major depressive disorder. J Mood Disord. 2015;5(4):145. [Crossref]
22. Zhang H, Ding L, Shen T, Peng D. HMGB1 involved in stress-induced depression and its neuroinflammatory priming role: a systematic review. Gen Psychiatry. 2019;32(4):1–9. [Crossref]
23. Yilmaz N, Yelboga Z, Yilmaz Y, Demirpence O. High mobility group box-1 levels in schizophrenia: potential biomarker of remission phase. J Med Biochem. 2020;1–7. [Crossref]
24. Al-Dujaili AH, Mousa RF, Al-Hakeim HK, Maes M. High mobility group protein 1 and Dickkopf-related protein 1 in schizophrenia and treatment-resistant schizophrenia: associations with interleukin-6, symptom domains, and neurocognitive impairments. Schizophr Bull. 2021;47(2):530–541. [Crossref]
25. Kozłowska E, Brzezińska-Błaszczyk E, Agier J, Wysokiński A, Żelechowska P. Alarmins (IL-33, sST2, HMGB1, and S100B) as potential biomarkers for schizophrenia. J Psychiatr Res. 2021;138:380–387. [Crossref]
26. Young, R. C., Biggs, J. T., Ziegler, V. E. & Meyer, D. A. Young Mania Rating Scale. Can J Clin Pharmacol.2004; 11: 540–542.
27. F. Karadağ, T. Oral, F. A. Yalçin, E. Erten. Reliability and validity of Turkish translation of Young Mania Rating Scale. Turkish journal of psychiatry. 2002;13:107–14.
28. Hamilton, M. A rating scale for depression. Journal of neurology, neurosurgery, and psychiatry.1960; 23:56–62.
29. Akdemir, A., Örsel, D.S., İşcan, N., Özbay, H., Dağ, İ.,Türkçapar, M.H. Hamilton depresyon derecelendirme ölçeği (HDDÖ)'nin geçerliliği- güvenirliliği ve klinikte kullanımı. Psikiyatri Psikoloji Psikofarmakoloji Dergisi. 1996;4:251–259.
30. Walker LE, Frigerio F, Ravizza T, Ricci E, Tse K, Jenkins RE, et al. Molecular isoforms of high-mobility group box 1 are mechanistic biomarkers for epilepsy. J Clin Invest. 2017;127(6):2118–2132. [Crossref]
31. Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Yolken R. Elevated C-reactive protein and cognitive deficits in individuals with bipolar disorder. J Affect Disord. 2013;150(2):456–459. [Crossref]
32. Wysokiński A, Margulska A, Strzelecki D, Kłoszewska I. Levels of C-reactive protein (CRP) in patients with schizophrenia, unipolar depression and bipolar disorder. Nord J Psychiatry. 2015;69(5):346–353. [Crossref]
33. Hsuchou H, Kastin AJ, Mishra PK, Pan W. C-reactive protein increases BBB permeability: Implications for obesity and neuroinfammation. Cell Physiol Biochem. 2012;30(5):1109–1119. [Crossref]
34. Jones BDM, Daskalakis ZJ, Carvalho AF, Strawbridge R, Young AH, Mulsant BH, et al. Inflammation as a treatment target in mood disorders: review. BJPsych Open. 2020;6(4):1–10. [Crossref]
35. Uyanik V, Tuglu C, Gorgulu Y, Kunduracilar H, Uyanik MS. Assessment of cytokine levels and hs-CRP in bipolar I disorder before and after treatment. Psychiatry Res. 2015;228(3):386–392. [Crossref]
36. Munkholm K, Jacoby AS, Lenskjold T, Bruunsgaard H, Vinberg M, Kessing LV. Leukocytes in peripheral blood in patients with bipolar disorder -trait and state alterations and association with levels of cytokines and C-reactive protein. Psychiatry Res. 2018;261:383–390. [Crossref]
37. Fusar-Poli L, Natale A, Amerio A, Cimpoesu P, Filioli PG, Aguglia E, et al. Neutrophil-to-lymphocyte, platelet-to-lymphocyte and monocyte-to-lymphocyte ratio in bipolar disorder. Brain Sci. 2021;11(1):1–10. [Crossref]
38. Netea MG, Quintin J, Van Der Meer JWM. Trained immunity: a memory for innate host defense. Cell Host Microbe. 2011;9(5):355–361. [Crossref]