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Evaluation of Changes in Peripheric Biomarkers Related to Blood Brain Barrier Damage in Patients with Schizophrenia and Their Correlation with Symptoms~~: A Case-Control Study~~

Running title: Peripheric Biomarkers Related to Blood Brain Barrier Damage in Schizophrenia

ABSTRACT

Objective: The aim of the study was to evaluate the levels of peripheric biomarkers that have been associated with blood brain barrier (BBB) damage in healthy controls and two groups of patients with schizophrenia, those who received typical-atypical antipsychotics and those who received only atypical antipsychotics. Additionally, we sought relationships between these biomarkers and schizophrenia symptoms.

Methods: This ~~case-control~~ study was conducted with the inclusion of 41 healthy volunteers and 75 patients with schizophrenia. The biomarkers measured to evaluate BBB injury were as follows: spectrin breakdown product 145 (SBDP145), spectrin breakdown product 150 (SBDP150), ubiquitin carboxy terminal hydrolase L1 (UCHL1), ubiquitin ligase cullin-3 (cullin), occludin and cl*a*~~*o*~~udin, which were measured via ELISA. Symptoms of patients with schizophrenia were evaluated with the Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS), the Clinical Global Impression Scale (CGI), and the general assessment of functionality (GAF).

Results: Compared to controls, SBDP145 (p~~P~~=0.022) and cullin (p~~P~~=0.046) levels were significantly higher in patients with schizophrenia receiving atypical antipsychotic treatment. SBDP150 levels were lower in the combination treatment group compared to the control group (p~~P~~=0.022). Claudin (p~~P~~=0.804), occludin (p~~P~~=0.058) and UCHL1 (p~~P~~=0.715) levels were similar among groups. In recipients of combination treatment, SBDP145 levels were found to be

positively correlated with SAPS-total (r=0.440, *p*~~P~~=0.036) and SAPS-delusions (r=0.494, *p*~~P~~=0.017) scores.

Conclusion: The relationships demonstrated in this study indicate that more comprehensive research is needed to understand whether BBB defects contribute to clinical characteristics in patients with schizophrenia.

KEY WORDS: Schizophrenia; Blood-Brain Barrier; Spectrin; Occludin; Claudins; Ubiquitin

INTRODUCTION

Schizophrenia is a chronic and severe mental disorder affecting 20 million people worldwide [1]. It manifests with a heterogeneous combination of symptoms which are classified as positive, negative and cognitive symptoms. Abnormal behaviors and thoughts, disorganized speech and behavior, delusions and hallucinations are considered as positive symptoms. Symptoms that include a motivational syndrome, social withdrawal, emotional flattening, anhedonia, reduced initiative and energy are negative symptoms [2].

Schizophrenia includes complex and closely interrelated mechanisms that affect inflammatory, oxidative, neurotransmitter and genetic pathways [3]. Although clinical evidence for blood brain barrier (BBB) impairment in patients with psychopathology is not definite, increased albumin levels in the CSF and increased S100B levels in serum strongly suggest the presence of BBB pathology in patients with schizophrenia [4]. The BBB consists of highly interconnected cells and is comprised of functional tight junctions, pericytes, surrounding basal lamina, extracellular matrix, neurovascular endothelial cells and perivascular astroglial processes. It has been reported that the hyperpermeability resulting from BBB defects may also contribute to the pathogenesis ~~[5].~~[4,5]. In the presence of BBB defects, the brain is exposed to harmful stressors which may lead to injury and could impair various functions [6]. Pollak *et al.* reported that the complex nature of BBB dysfunction in schizophrenia may be related to many aspects of the impaired neuronal and synaptic function, including increased permeability to inflammatory molecules, impaired glutamate homoeostasis, and non-response or resistance to antipsychotics [7]. Therefore, its integrity is critical for maintaining brain homeostasis and immune protection [3].

Postmortem studies of the limbic structures of patients with schizophrenia show various cytological architectural abnormalities that may result from abnormalities of neuronal cytoskeletal

proteins [8]. Neurodegeneration-related molecules, such as spectrin breakdown product 120 (SBDP120), spectrin breakdown product 145 (SBDP145), spectrin breakdown product 150 (SBDP150), claoudin, occludin and ubiquitin C-Terminal Hydrolacase-L1 (UCHL1), are reported to be associated with BBB [3,8-14]. Moreover, the dose-dependent expression of claudin-5 in rats was found to be a modifying factor in schizophrenia [10]. Considering that such alterations may result in changes in the BBB and drug passage to the brain, it is evident that there is a need for studies evaluating BBB-related damage in patients with schizophrenia [15] . [7,15]. However, data on serum biomarker levels associated with BBB in patients with schizophrenia using typical/atypical antipsychotics are limited. Therefore, the current study aimed to examine the biomarkers of BBB injury and their relationships with clinical characteristics or treatment in patients with schizophrenia.

METHODS

This cross-sectionalase control study was carried out in Corum Community Mental Health Center and Hitit University Erol Olcok Training and Research Hospital between June 2019 and March 2020. Ethics committee approval was obtained from the Clinical Ethics Committee of 19 Mayıs University Faculty of Medicine for the study (No: 2019/06, Date: 18.03.2019). The research was carried out in accordance with the Declaration of Helsinki.

Patients between the ages of 18-70 who attended follow-up in Corum Community Mental Health Center with a diagnosis of schizophrenia (according to the Structured Clinical Interview for DSM-V Axis I, SCID I) for at least one year were included to this study. Among these patients, those who had received only atypical antipsychotics or combination treatment (typical-atypical antipsychotics) for the last 6 months, and agreed to participate in the study (approval obtained from the patients themselves, if applicable, or from legal guardians) were included in the study

group. Patients who had psychiatric axis I comorbidity, those with additional neurological disease or organic brain disorders such as degenerative disease, epilepsy, central nervous system tumor or cerebrovascular disease that could affect neurocognitive tests, subjects who had undergone electro convulsive therapy (ECT) within the last 6 months, patients with a history of head trauma leading to unconsciousness, mental retardation, acute psychotic symptoms requiring hospitalization during the study, those with a history of substance/alcohol abuse or serious physical disease, and individuals who did not agree to participate in the study were excluded. A total of 75 patients with schizophrenia (50 used only atypical antipsychotics and 25 used combination treatment) were included in the study. The control group consisted of 41 individuals employed at Hitit University Erol Olcok Training and Research Hospital, who were similar to the patients in terms of age, gender and educational status, and who were found to have no psychiatric disorder according to SCID-I. Patients and controls meeting the study criteria were given detailed information about the purpose and scope of the study. Written and verbal consent was obtained from those who accepted to participate in the study. The sociodemographic data form and a questionnaire consisting of scales were administered to the research group by the physicians conducting the research. After completion of this initial step, blood samples were taken from the patients and controls.

Scale for the Assessment of Positive Symptoms (SAPS)

The scale used to measure the level, distribution and severity of the positive symptoms of schizophrenia was developed by Andreasen. It is based on the information obtained during the interview with the patient and their relatives, and the observations during the interview. The scale includes four sub-dimensions (hallucinations, delusions, bizarre behavior, positive formal thought disorder) measured via 34 six-point Likert-type items. As the score obtained from the scale

increases, the severity of the patient's positive symptoms increases. The Turkish validity and reliability study of the scale was conducted by Erkoc *et al*. [16,17].

Scale for the Assessment of Negative Symptoms (SANS)

The scale used to measure the level, distribution and severity of the negative symptoms of schizophrenia was developed by Andreasen. The scale consists of five sub-dimensions (blunting, alogia, avolition, anhedonia and attention) measured via 25 six-point Likert-type items. The validity and reliability of the Turkish form of the scale was demonstrated by Erkoc *et al*. [16,18].

Clinical Global Follow-up Scale (CGI)

The CGI was used to evaluate the functionality of the individuals. The scale, which enables the evaluation of functionality of the patient before and after treatment, was developed by Guy in 1976. There are three sub-dimensions in the scale that evaluate the severity of the disease, recovery and drug side effects [19].

General assessment of functionality (GAF)

The GAF scale was used to evaluate the psychological, social and professional functionality of the individuals. The scale allows functionality at the time of measurement or in the past, both are rated with a score ranging from 1 to 100. High scores on the scale indicate high functionality [20].

Laboratory measurements

The blood samples of the study group were taken into vacuum tubes containing ethylene diamine tetra acetic acid (EDTA) after at least 10 hours of fasting. After waiting half an hour at room temperature, blood samples were centrifuged at 2.683 rcf for 10 minutes. Centrifuged blood samples were stored at -30 ° C until protein / enzyme analyzes associated with BBB damage were performed with sandwich-ELISA kits (Bioassay Technology Laboratory, Shanghai, China).~~Blood~~

~~samples were taken into the tubes without anticoagulant and with ethylene diamine tetra acetic acid, after 12 hours of fasting for serum and plasma, respectively. The blood samples were kept at room temperature for half an hour, and were then centrifuged at 4000×rpm for 10 minutes, and then stored at -30 °C until the analyses of proteins/enzymes associated with BBB injury were performed with sandwich ELISA kits.~~ Samples were added to respective commercial kits designed for the detection of SBDP145 (sensitiviy:0.042 ng/mL, detection range:0.1-40 ng/mL, intra-Assay: CV<%8), SBDP150 (sensitiviy:0.117 ng/mL, detection range:0.2-60 ng/mL, intra-Assay: CV<%8), UCHL1 (sensitiviy:0.056 ng/mL, detection range:0.1-38 ng/mL, intra-Assay: CV%<8%), cullin (sensitiviy:7.83 ng/mL, detection range:1.5-300 ng/mL, intra-Assay: CV%<8%), occludin (sensitiviy:0.27 ng/mL, detection range:0.5-150 ng/mL, intra-Assay: CV%<8%) and cl~~a~~a~~o~~udin (sensitiviy:0.22 ng/mL, detection range:0.5-40 ng/mL, intra-Assay: CV%<8%) in human samples. All measurements were performed with respect to the manuals provided by kit manufacturers (Bioassay Technology Laboratory, Shanghai, China). After all necessary steps, absorbance measurements were performed at 450 nm wavelength with ELISA reader (SPECTRA MAX 190 ELISA Washer and Reader). Using the optical density and concentration values of the standards, the levels of all parameters were determined in each sample.

Statistical analysis

All analyses were performed on SPSS v15 (SPSS Inc., Chicago, IL, USA). Number, percentage, mean, standard deviation, median, minimum and maximum values were used in the evaluation of descriptive data. The Shapiro-Wilk test was used to determine whether the continuous variables conformed to the Gaussian distribution. The Chi-square (for categorical variables), Mann Whitney U and Kruskal Wallis tests (for continuous variables) were used for comparisons between and

among groups. Correlations between biomarker levels and scores were evaluated with the Spearman correlation analysis. Statistical significance level was accepted as ≤0.05.

RESULTS

The ages of the individuals constituting the study group ranged from 25 to 66, with a mean of 45.5±9.2 years. Patient and control groups were similar in terms of age (p= 0.766) and gender distribution (p= 0.603) (**Table 1**). Among the schizophrenic patients, the median age of those who received atypical antipsychotic treatment was significantly lower compared to those receiving combination treatment (p= 0.012). It was found that schizophrenia patients receiving combination treatment or atypical antipsychotic treatment were similar in terms of other characteristics (**Table 2**).

The GAF scale scores were found to be similar between the combination treatment and atypical antipsychotic treatment groups (p= 0.063). The median score obtained from CGI was found to be significantly higher in patients who received combination treatment (p= 0.007). The median SANS-blunting (p= 0.030), SANS-alogia (p= 0.009), SANS-anhedonia (p= 0.049) and SANS-total (p= 0.026) scores were found to be significantly lower in patients receiving atypical antipsychotic treatment. The scores from the SANS-avolution (p= 0.353) and SANS-attention (p= 0.167) sub-domains were similar between the two groups. It was found that the SAPS-hallucinations (p= 0.423), SAPS-delusions (p= 0.932) and SAPS-total (p= 0.808) scores did not differ according to the type of antipsychotic treatment. SAPS-bizarre behavior (p= 0.038) and SAPS-positive formal thought disorder (p= 0.005) sub-dimensions were found to be significantly higher in those who received atypical antipsychotic treatment (**Table 3**).

SBDP145 level was significantly higher in patients with schizophrenia receiving atypical antipsychotic treatment compared to controls (p= 0.022). SBDP150 level was significantly lower

in patients with schizophrenia receiving combination treatment compared to the control group (*p*~~P~~= 0.022). Cullin level was significantly higher in patients with schizophrenia using atypical antipsychotics compared to the healthy controls (*p*~~P~~= 0.046). Claudin (*p*~~P~~= 0.804), occludin (*p*~~P~~= 0.058) and UCHL1 (*p*~~P~~= 0.715) levels were found to be similar between the groups (**Table 4**~~.~~ **Figure 1-3**).

A positive correlation was found between SAPS-total score and SBDP145 level in patients with schizophrenia receiving combination treatment (r= 0.440, *p*~~P~~= 0.036). When the subdomain scores were analyzed in this group, it was found that the SAPS-delusions sub-domain score was positively correlated with SBDP145 level (r= 0.494, *p*~~P~~= 0.017). No other statistically significant correlations were found between clinical scores and biomarker levels (**Table 5**).

DISCUSSION

The mechanisms underlying the development and progression of psychiatric diseases have not been elucidated yet. In psychotic disorders, evidence suggesting the presence of BBB dysfunction is gradually increasing, and considering the functions of the BBB, barrier dysfunction is likely to contribute to psychosis pathology~~.~~ [5,7]~~.~~ ~~.~~ [4,5,7]. This is the first ~~case-control~~ study, in which biomarkers of SBDP145, SBDP150, UCHL1, cullin, occludin and cl*a*~~o~~udin that are associated with BBB injury, and their relationship with symptoms in patients with schizophrenia receiving atypical or typical/atypical antipsychotic treatment were evaluated.

Spectrin proteins are enriched constituent cellular components that have many important biological roles in membrane stability, dynamics and remodeling. Spectrin breakdown products in the cerebrospinal fluid (CSF) and brain may demonstrate the calpain- and caspase-mediated mechanisms of neuronal cell death, and therefore, these could be used as biomarkers to predict the development of brain damage. In traumatic and ischemic brain lesions, calcium-induced activation

of calpain and caspase-3 causes excess spectrin proteolysis [8,13,14]. For instance, Mondello *et al*. found that SBDP150, SBDP145 and SBDP120 levels demonstrated variations after time in patients with traumatic brain injury [21]. With regard to the studies focused on patients with schizophrenia, the study by Kitamura *et al*. showed an increase in the 150 kDa form of α-spectrin in the left upper temporal cortex of schizophrenic brains compared to controls in postmortem analyses [22]. On the other hand, Murakami *et al*. reported that α-spectrin gene polymorphisms do not play an important role in the genetic component of schizophrenia [23]. In the study of Cetin and Demirel, it was reported that serum SBDP120 and SBDP145 levels were significantly higher in patients with schizophrenic when compared to healthy controls [24]. In the present study, although we found SBDP145 levels to be significantly higher in patients with schizophrenia compared to controls, SBDP150 levels were significantly lower than controls. When taken together, it is possible to conclude that the BBB injury in patients with schizophrenia may be associated with specific pathways which result in the release of different forms of SBDP. However, it is evident that this conclusion must be evaluated with caution in future studies. Furthermore, the lack of longitudinal assessments is an important limitation in this respect, since disease duration, treatment type and response, and various other characteristics may alter the levels of these markers throughout the disease process.

Tight junctions are at the center of a dynamic and highly regulated BBB system by preventing the flow of solutes and ions from blood to the brain and vice versa. The tight junctions include claudines and occludin, which are bound to the actin cytoskeleton by the zonula occludense (ZO) proteins [25]. The number of studies on occludin in patients with schizophrenia is limited. In a study in which postmortem tissue examination was performed, it was reported that the level of protein mRNA originating from occludin was found to be high in patients with schizophrenia,

possibly in relation with inflammatory activation [26]. In another postmortem tissue analysis among patients with depression, it was reported that occludin level was high in the occipital cortex [27]. In our study, occludin levels were found to be similar between the groups. Claudine, the primary sealing component of the tight junction, is an important BBB component and is crucial for angiogenesis and endothelial maintenance [28,29]. Claudin 5 polymorphism has been reported to be associated with schizophrenia [30]. Additionally, experimental studies have shown that the loss of claudin-5 in the rat brain is associated with schizophrenia-related behaviors [10]. Greene *et al*. reported that claudin-5 expression decreased in the hippocampus of patients with schizophrenia [27]. In the study of Cai *et al.*, claudine proteins were also found to be elevated in the postmortem tissue of patients with schizophrenia [26]. In the present study, the groups were found to be similar in terms of claudine levels. However, it is known that antipsychotic drugs may increase the expression of claudine-5 in a dose-dependent manner in vitro and in vivo [10], which may have led to the lack of difference between the groups in this study.

The ubiquitin proteasome system is a highly complex system that has important roles in various basic cellular processes, particularly in the breakdown of intracellular proteins [31]. In previous studies, the decline of ubiquitin degradation products was associated with schizophrenia [32-34]. In the current study, while there was no statistically significant difference between the patients with schizophrenia and control groups in terms of UCHL1 levels in relation to antipsychotic treatment, we found that cullin level was higher in patients with schizophrenia receiving atypical antipsychotic treatment when compared to healthy controls. The possibility that previous metabolic changes in patients with schizophrenia are partially normalized by the therapeutic effect of antipsychotic treatment [33], may explain the difference in outcomes.

Abnormalities in the BBB of patients with schizophrenia can affect the cognitive and behavioral symptoms of patients, in addition to the clinical course and treatment response, through various mechanisms including decreased cerebral perfusion and homeostatic processes of the cerebral microenvironment ~~[3].~~ [3,7]. Muller and Ackenhei reported that the CSF-to-serum albumin ratio was associated with the negative symptomatology in schizophrenia, and patients with albumin-related changes may have a higher risk of developing the negative symptoms [35]. In the study of Çetin and Demirel, it was reported that there was a positive correlation between serum SBDP120 levels and scores related to the positive and negative symptoms in patients with schizophrenia [24]. In partial conflict, Bousman *et al.*, found that there was no relationship between UCHL1 and cullin levels and the scores of the SAPS and SANS scales [36]. In the present study, we found a positive correlation between the SBDP145 and SAPS total score and SAPS-delusion score in those receiving combination antipsychotic treatment. The fact that there is a correlation between BBB biomarkers and symptoms means that biomarker changes caused by the dysfunction of the BBB can also affect the symptoms of schizophrenia. Even though these results require confirmation in future studies, we believe that current evidence demonstrates the need for new therapeutic methods that could aid in the approach to treatment of schizophrenia, especially with respect to the repair of dysfunctional BBB.

One of the limitations of the study is that the median age of the group treated with atypical antipsychotics was lower compared to the group with combination treatment. This difference may have affected some results. The low number of patients in the study group is another limitation. As mentioned before, the study does not include long-term periodic measurements including various stages of disease. However, in order to be able to normalize the characteristics of patients, we included patients with at least one year disease history and had been receiving treatment for at

least six months. Nonetheless, regular evaluations from the onset of schizophrenia could provide important evidence on the development and progression of BBB dysfunction, and the chronology of the relationship between BBB dysfunction and schizophrenia symptoms which could provide evidence for causal assessments. Moreover, a study planned in this manner could enable assessment of the possible toxic effects and consequences of antipsychotic therapy on BBB [37]. Despite these limitations, this study is interesting in that it is the first study examining the relationship between schizophrenia and BBB injury with the measurement of SBDP145, SBDP150, UCHL1, cullin, occludin and claudin.

Conclusions

The levels of SBDP145 and cullin, as measures of BBB injury, were found to be significantly higher in patients with schizophrenia receiving atypical antipsychotic treatment compared to controls. However, SBDP150 level was significantly lower in patients receiving combination antipsychotic treatment compared to healthy controls. There was no difference between the groups in terms of claudin, occludin and UCHL1 levels. The relationships shown between symptom scores and biomarker levels could be useful when designing future studies on this topic, since the positive correlation between SBDP145 levels and SAPS-total and SAPS-delusion scores in patients with combination treatment raises questions, not only with respect to the relationship between schizophrenia and BBB injury, but also in terms of possible toxic effects of medications on the BBB. Biomarkers of BBB injury appear to show remarkable changes in patients with schizophrenia, and these markers may in fact be associated with symptoms. More comprehensive studies including short and long-term evaluation of BBB injury in patients with schizophrenia are needed to elucidate the existence of causal relationships between BBB markers and patient symptoms.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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FIGURE LEGENDS

Figure 1. SBDP145 levels of groups

Figure 2. SBDP150 levels of groups

Figure 3. Cullin levels of groups

Table 1. Comparison of age and gender of patients with schizophrenia and healthy control group

	Schizophrenia n=75	Healthy control n=41	Total n=116	*p*
Age, median (min-max)	45 (25-66)	44 (27-60)	44 (25-66)	0.766
Gender, n (%)				
Male	44 (58.7)	22 (53.7)	66 (56.9)	0.603
Female	31 (41.3)	19 (46.3)	50 (43.1)	

Table 2. Basic characteristics of patients with schizophrenia according to treatment method

	Typical-atypical antipsychotic group n=25	Atypical antipsychotic group n=50	Overall n=75	*p*
Age	50.0 (29.0-65.0)	40.5 (25.0-66.0)	45.0 (25.0-66.0)	0.012
Body mass index, (kg/m^2)	29.9 (18.7-46.6)	30.6 (20.2-41.6)	30.4 (18.7-46.6)	0.399
Disease onset age	22.0 (8.0-48.0)	21.0 (13.0-60.0)	21.0 (8.0-60.0)	0.770
First treatment age	26.0 (8.0-48.0)	24.5 (13.0-60.0)	25.0 (8.0-60.0)	0.347
Number of hospitalizations	5.0 (0.0-20.0)	3.0 (0.0-35.0)	3.0 (0.0-35.0)	0.199
Gender				
Male	16 (64.0)	28 (56.0)	44 (58.7)	0.507
Female	9 (36.0)	22 (44.0)	31 (41.3)	
Marital status				
Not married	18 (72.0)	29 (58.0)	47 (62.7)	0.237
Married	7 (28.0)	21 (42.0)	28 (37.3)	
Employment status				
Not working / retired	24 (96.0)	43 (86.0)	67 (89.3)	0.186
Working	1 (4.0)	7 (14.0)	8 (10.7)	
Education status				
Primary school	21 (84.0)	34 (68.0)	55 (73.3)	
High school	3 (12.0)	12 (24.0)	15 (20.0)	0.336
University	1 (4.0)	4 (8.0)	5 (6.7)	
Alcohol use				

Absent	24 (96.0)	47 (94.0)	71 (94.7)	0.716
Present	1 (4.0)	3 (6.0)	4 (5.3)	
Smoking				
Absent	9 (36.0)	23 (46.0)	32 (42.7)	0.409
Present	16 (64.0)	27 (54.0)	43 (57.3)	
Suicide history				
Present	3 (12.0)	13 (26.0)	16 (21.3)	0.163
Absent	22 (88.0)	37 (74.0)	59 (78.7)	
Schizophrenia in the family				
Present	6 (24.0)	8 (16.0)	14 (18.7)	0.402
Absent	19 (76.0)	42 (84.0)	61 (81.3)	
Affective disorder in the family				
Present	4 (16.0)	16 (32.0)	20 (26.7)	0.140
Absent	21 (84.0)	34 (68.0)	55 (73.3)	

Results were presented as median (min-max) and n (%) for continuous and categorical variables, respectively.

Table 3. Comparison of positive and negative symptoms of patients with schizophrenia by type of treatment

	Typical-atypical antipsychotic group n=25	Atypical antipsychotic group n=50	Overall n=75	p
GAF	48 (31-70)	50 (30-80)	50 (30-80)	0.063
CGI	9 (4-10)	7.5 (3-10)	8.0 (3.0-10)	0.007
SANS-Blunting	22 (1-32)	14 (0-36)	16 (0-36)	0.030
SANS-Alogia	8 (0-19)	4 (0-17)	6 (0-19)	0.009
SANS-Avolition	5 (0-14)	3 (0-15)	4 (0-15)	0.353
SANS-Anhedonia	14 (0-20)	10 (0-23)	11 (0-23)	0.049
SANS-Attention	7 (0-14)	6 (0-15)	6 (0-15)	0.167
SANS-Total	56 (8-95)	37 (4-95)	43 (4-95)	0.026
SAPS- Hallucinations	2 (0-18)	1 (0-17)	1 (0-18)	0.423
SAPS-Delusions	3 (0-16)	3.5 (0-18)	3 (0-18)	0.932
SAPS-Bizarre behavior	0 (0-1)	0 (0-5)	0 (0-5)	0.038
SAPS-Positive formal thought disorder	0 (0-26)	0 (0-22)	0 (0-26)	0.005
SAPS-Total	9 (0-60)	9.5 (0-32)	9 (0-60)	0.808

Results were presented as median (min-max). GAF: General assessment of functionality, CGI: Clinical Global Follow-up Scale, SANS: Scale for the Assessment of Negative Symptoms, SAPS: Scale for the Assessment of Positive Symptoms.

Table 4. Distribution of BBB injury biomarkers in schizophrenia patients and healthy controls

	Typical-atypical antipsychotic group n=25	Atypical antipsychotic group n=50	Healthy control n=41	*p*
SBDP145 (ng/mL)	4.9 (4.0-19.8)	5.1 (3.7-31.4)[*]	4.3 (2.3-14.3)[*]	0.022
SBDP150 (ng/mL)	6.5 (4.4-18.1)[*]	6.9 (4.1-30.1)	8.7 (5.1-32.2)[*]	0.022
UCHL1 (ng/mL)	4.0 (2.9-27.6)	4.4 (3.0-28.9)	4.5 (2.7-24.6)	0.715
Cullin (ng/mL)	380 (280-2746)[ab]	420 (266-2824)[a]	368 (201-1182)[b]	0.046
Occludin (ng/mL)	12.3 (8.5-31.0)	12.1 (8.9-52.1)	15.7 (9.8-73.2)	0.058
Claudin (ng/mL)	4.7 (3.6-45.5)	4.9 (3.6-55.4)	5.6 (2.8-35.3)	0.804

Results were presented as median (min-max). SBDP145: Spectrin breakdown product 145, SBDP150: Spectrin breakdown product 150, UCHL1: Ubiquitin C-Terminal Hydrolacase-L1

Table 5. Correlations of BBB injury biomarkers with SAPS, SANS, CGI and GAF scores

	SAPS-Total (r/*p*)	SANS-Total (r/*p*)	CGI (r/*p*)	GAF (r/*p*)
Atypical antipsychotic group				
SBDP145 (ng/mL)	0.089/0.537	0.086/0.550	0.008/0.957	0.016/0.911
SBDP150 (ng/mL)	-0.055/0.711	-0.013/0.931	0.052/0.724	0.156/0.290
UCHL1 (ng/mL)	0.119/0.410	0.111/0.442	0.063/0.662	-0.023/0.872
Cullin (ng/mL)	0.127/0.386	0.115/0.430	0.017/0.908	-0.035/0.810
Occludin (ng/mL)	0.063/0.675	0.206/0.164	0.063/0.675	-0.073/0.627
Claudin (ng/mL)	0.125/0.386	0.075/0.606	0.190/0.186	-0.027/0.853
Typical-atypical antipsychotic group				
SBDP145 (ng/mL)	0.440/0.036	-0.06/0.785	0.07/0.750	0.003/0.990
SBDP150 (ng/mL)	0.215/0.337	-0.223/0.318	-0.091/0.688	0.097/0.667
UCHL1 (ng/mL)	0.351/0.100	-0.094/0.671	-0.014/0.948	-0.029/0.894
Cullin (ng/mL)	0.239/0.284	-0.109/0.631	0.139/0.539	-0.072/0.751
Occludin (ng/mL)	0.298/0.178	-0.120/0.596	0.004/0.988	-0.040/0.860
Claudin (ng/mL)	-0.042/0.849	-0.125/0.568	0.084/0.703	-0.048/0.828
Total				
SBDP145 (ng/mL)	0.199/0.092	0.021/0.858	-0.004/0.973	0.033/0.781
SBDP150 (ng/mL)	0.063/0.607	-0.109/0.367	-0.043/0.726	0.175/0.148
UCHL1 (ng/mL)	0.185/0.117	0.020/0.864	-0.013/0.914	0.013/0.915
Cullin (ng/mL)	0.175/0.145	0.004/0.974	0.022/0.858	-0.017/0.888
Occludin (ng/mL)	0.151/0.217	0.062/0.614	0.008/0.951	-0.026/0.832

Claudin (ng/mL)	0.089/0.452	-0.016/0.891	0.129/0.276	-0.021/0.860

GAF: General assessment of functionality, CGI: Clinical Global Follow-up Scale, SANS: Scale for the Assessment of Negative Symptoms, SAPS: Scale for the Assessment of Positive Symptoms, SBDP145: Spectrin breakdown product 145, SBDP150: Spectrin breakdown product 150, UCHL1: Ubiquitin C-Terminal Hydrolacase-L1.

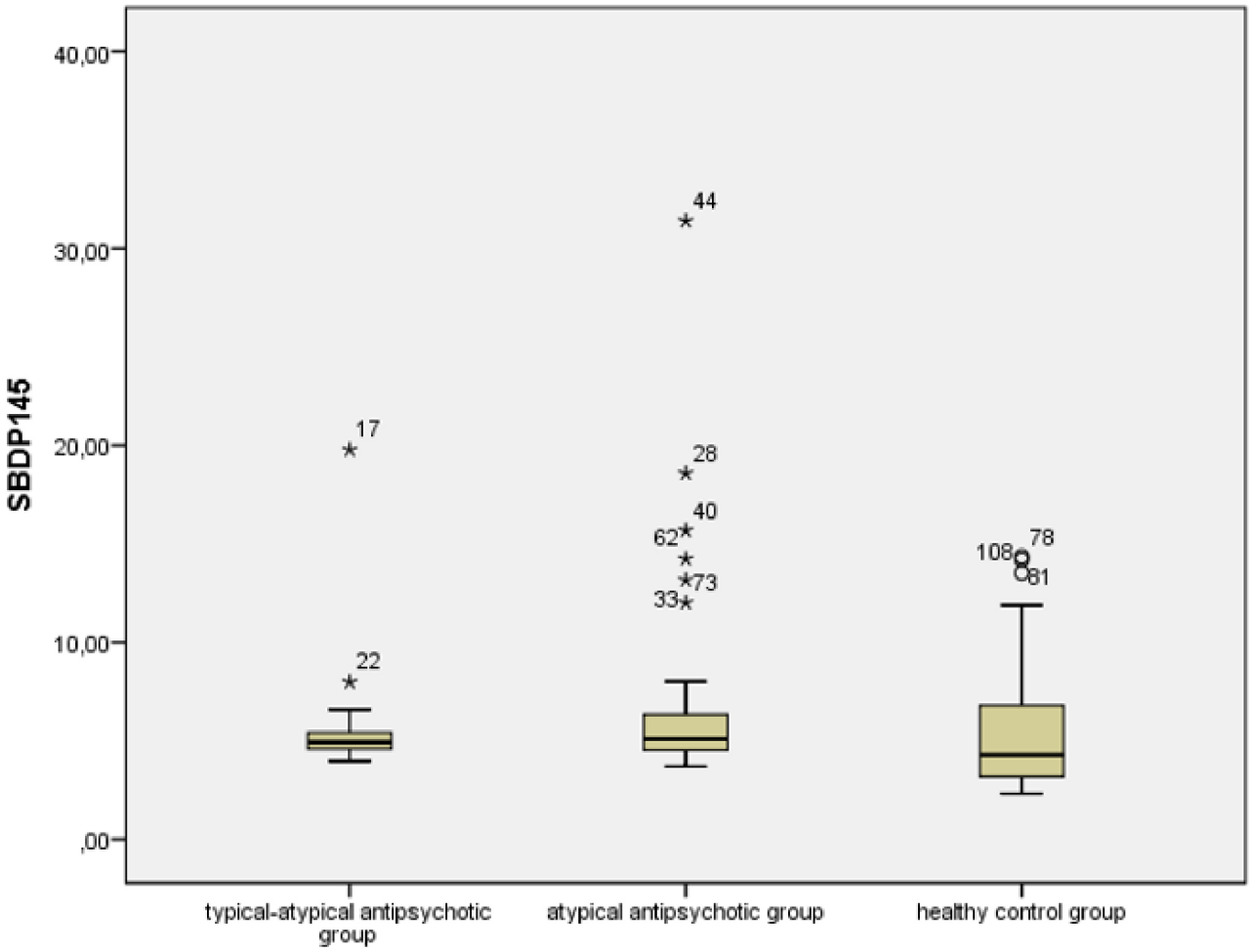

Fig. 1. SBDP145 levels of groups

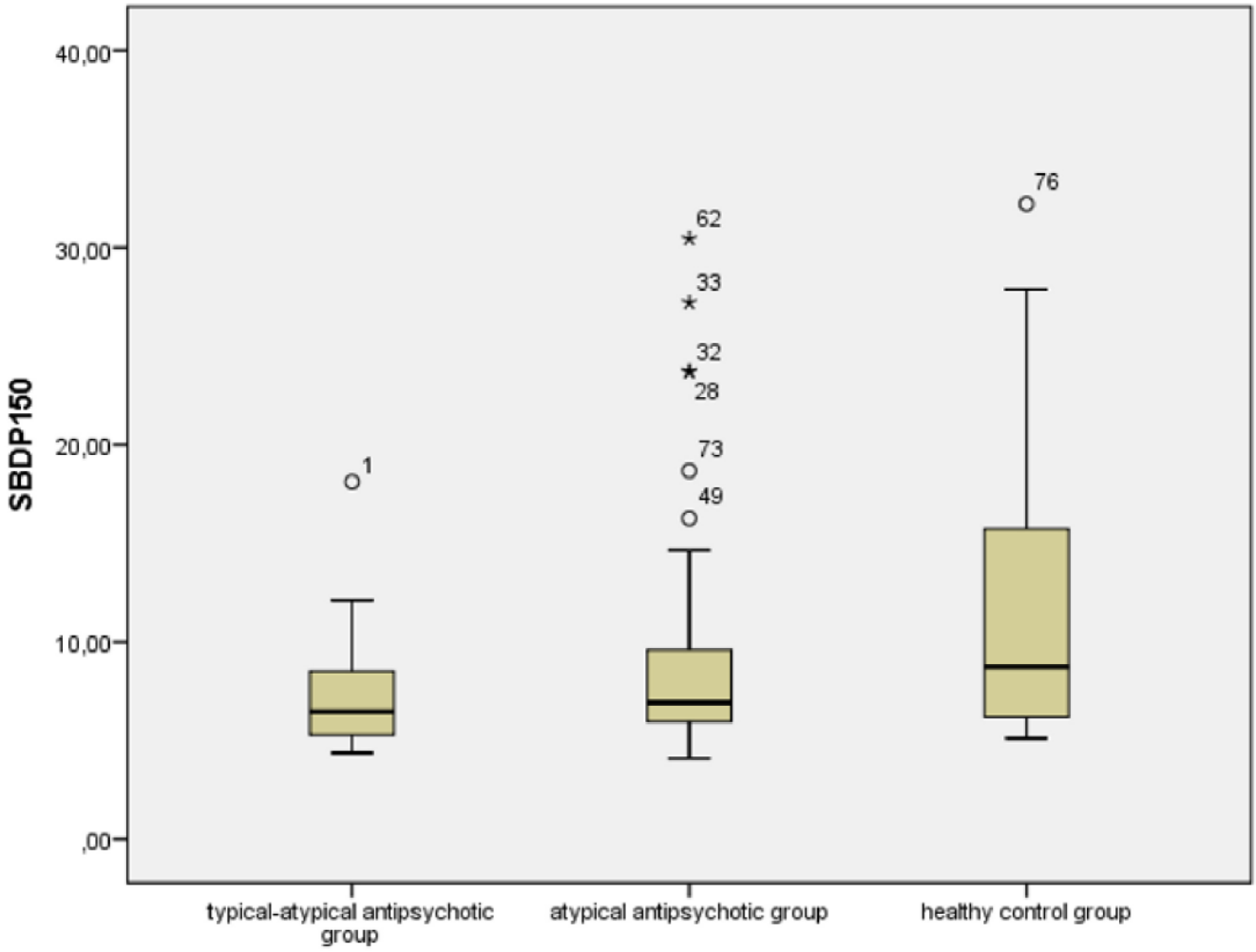

Fig. 2. SBDP150 levels of groups

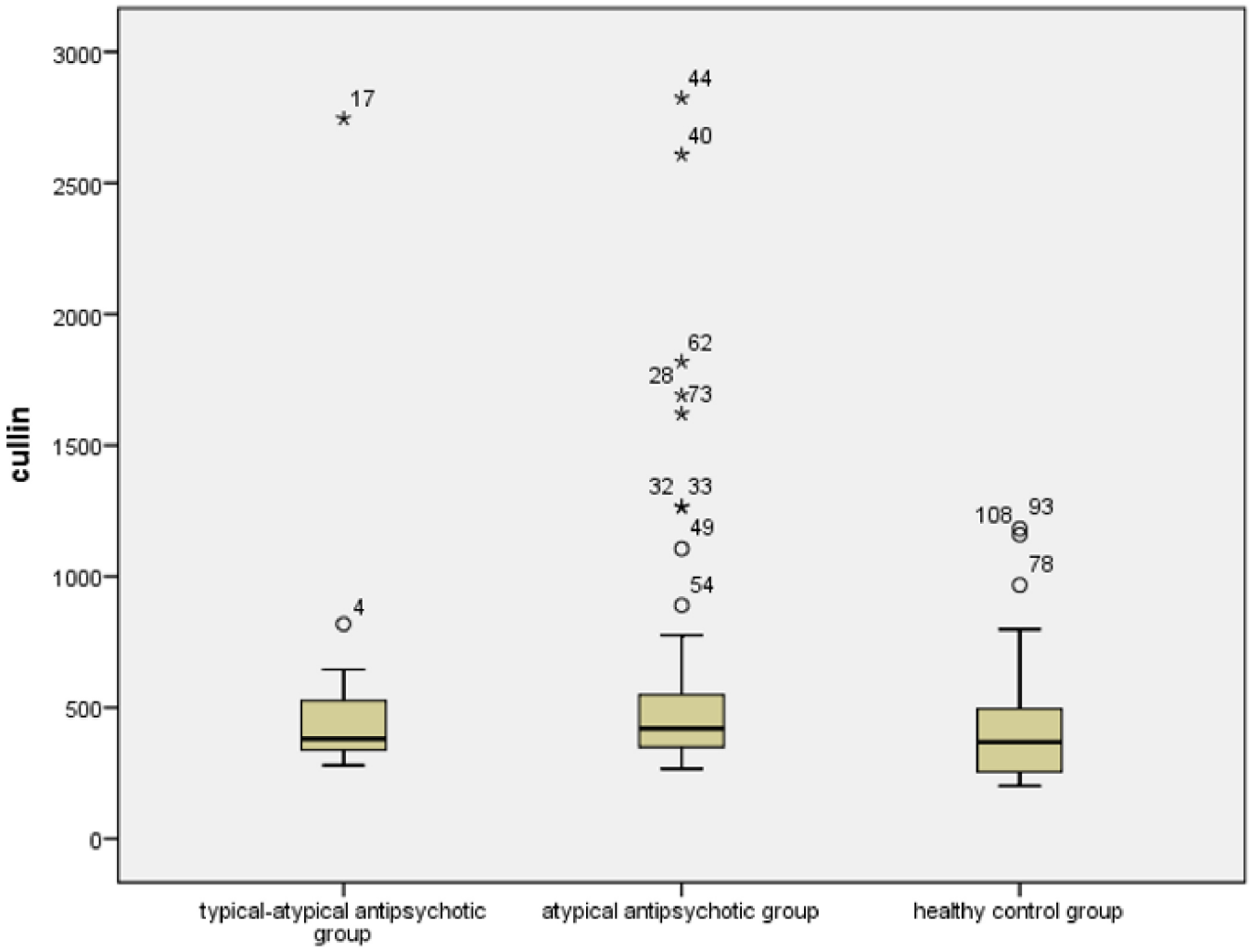

Fig. 3. Cullin levels of groups