Evaluation of Serum Complement Levels and Factors Affecting Treatment Resistance in Patients with Schizophrenia

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ABSTRACT

Background: There are increasing investigations about the potential role of the complement system in disorders affecting the central nervous system, including schizophrenia. Therefore, we aim to evaluate the levels of complement 3 and complement 4 and the factors affecting treatment resistance in schizophrenia patients.

Methods: This cross-sectional study was conducted between January 2020 and January 2021 and included schizophrenia patients resistant to treatment or in remission and healthy controls. The Structured Clinical Interview for Diagnostic and Statistical Manual-5 was used to confirm the diagnosis according to Diagnostic and Statistical Manual -5 criteria. We evaluated the patients with some scales and forms. The complement 3 and complement 4 levels were measured from blood samples.

Results: In the treatment-resistant schizophrenia group, complement 3 ($P = .001$) and complement 4 ($P = .001$) levels were significantly higher compared to schizophrenia patients in remission and healthy controls. While the Brief Psychiatric Rating Scale ($P < .001$), the Positive and Negative Syndrome Scale—positive ($P < .001$), the Positive and Negative Syndrome Scale—negative ($P < .001$), the Positive and Negative Syndrome Scale—psychopathology ($P < .001$), the Positive and Negative Syndrome Scale—total ($P < .001$), and the Clinical Global Impression Scale—Severity ($P < .001$) scores were significantly higher in treatment-resistant schizophrenia patients, the General Assessment of Functioning ($P < .001$), and Beck Cognitive Insight Scale ($P < .001$) scores were significantly lower compared to the other groups. In schizophrenia patients, complement 3 levels were positively correlated with the Positive and Negative Syndrome Scale—negative ($P = .046$), the Positive and Negative Syndrome Scale—psychopathology ($P = .001$), the Positive and Negative Syndrome Scale —total ($P = .025$), and Clinical Global Impression Scale—Severity of Disease ($P = .004$). Also, complement 4 levels were positively correlated with Brief Psychiatric Rating Scale ($P = .004$), the Positive and Negative Syndrome Scale—positive ($P = .003$), the Positive and Negative Syndrome Scale —negative ($P = .014$), the Positive and Negative Syndrome Scale—psychopathology ($P < .001$), the Positive and Negative Syndrome Scale—total ($P = .002$), and Clinical Global Impression Scale—Severity of Disease ($P = .001$) in patients with schizophrenia. It was determined that a higher C4 level increased the risk of treatment resistance (odds ratio: 1.133, 95% CI: 1.012-1.268; $P = .030$), while a higher Beck Cognitive Insight Scale score decreased the risk of treatment resistance (odds ratio: 0.317, 95% CI: 0.191-0.526; $P < .001$).

Conclusion: In light of the analyses, it can be said that complement concentration increases in certain stages of schizophrenia, and its imbalance may be associated with symptom severity and treatment resistance.

INTRODUCTION

Schizophrenia is a chronic psychiatric disorder that usually begins in the early twenties, is characterized by cognitive, perceptive, and motivational disorders, and has negative effects on both patients and the society.[1] The lifetime prevalence of schizophrenia is reported to be 5.5 per 1000 people worldwide and 8.9 per 1000 people in Turkey.[2,3]

In schizophrenia, signs, symptoms, response to treatment, and course of the disease vary among patients.[4] For symptomatic remission, maintenance over a 6-month period in which specific symptoms are less severe and high functioning is needed.[5] Approximately 1 in 2 schizophrenia patients relapse after the first psychotic episode despite using a regular medication, and as the number of episodes increases, the likelihood of chronicity also increases.[1] Although the studies have used a variety of different approaches to defining treatment resistance,

treatment-resistant schizophrenia can be briefly defined when a patient is unresponsive to certain treatments over a period of time.[5]

Although the pathophysiology of schizophrenia has not yet been fully elucidated, the number of studies on the role of the immune system in this disease is increasing.[6-8] The fact that some schizophrenia patients have a history of hospitalization due to infection before the onset of the disease, a history of infection in the prenatal and childhood periods, and an increase in inflammatory markers in schizophrenia patients suggest that the immune system may be associated with schizophrenia.[9,10] A meta-analysis examining the results of 41 studies evaluating postmortem brain tissue reported a significant increase in microglia and proinflammatory gene expression in the postmortem brains of schizophrenia patients compared to controls.[11] While prenatal inflammation is associated with an increased risk of schizophrenia, it has been reported that molecular markers of inflammation such as peripheral cytokines, chemokines, and acute phase reactants are increased in patients with schizophrenia and their first-degree relatives.[9] The complement system consists of more than 30 proteins that contribute to host defense against microorganisms, antibody-mediated tissue damage, identification of injured tissues, and clearance of cellular debris. Activation of the complement pathway results in inflammatory and immune responses[12] and leads to a series of enzymatic reactions that initiate physiological responses such as chemotaxis and apoptosis.[13] It has been reported that proteins in the complement system have an important role in the pathogenesis of schizophrenia through genetic, inflammatory, infectious, and autoimmune processes.[14-16] Again a growing number of studies have recently been published using anti-inflammatory drugs in schizophrenia patients to find alternative treatment procedures for patients who have not responded to conventional antipsychotic treatment.[17]

Schizophrenia researches regarding complement changes are inconsistent. For example, a study by Wong et al[18] on alterations of some serum acute phase proteins in patients with schizophrenia reported that the C3 concentration decreases in chronically ill schizophrenic patients, while Sória et al[19] reported increased plasma levels of C3 and C4.

MAIN POINTS

- Complement 3 (C3) and C4 levels were higher in treatment-resistant schizophrenia patients compared to those in remission and also healthy controls.
- C3 and C4 levels were correlated with symptom severity in patients with schizophrenia.
- Higher C4 levels and lower Beck Cognitive Insight Scale scores were found to increase the likelihood of treatment resistance in schizophrenia.

Despite these results, there are very few studies evaluating possible relationships between the complement system, symptom severity, and treatment resistance in patients with schizophrenia. We hypothesized that the levels of C3 or C4 would be positively associated with symptom severity and treatment resistance in schizophrenia. In this context, the aim of the present study was to evaluate the relationship of C3 and C4 levels with symptom severity and treatment resistance in patients with schizophrenia.

MATERIALS AND METHODS

Patient Selection

This cross-sectional study was conducted between January 2020 and January 2021 at the Bakırköy Prof. Dr. Mazhar Osman Training and Research Hospital for Psychiatry, Neurology and Neurosurgery clinics and Community Mental Health Centers affiliated to this institution. In order to carry out the research, ethics committee approval from the Bakırköy Prof. Dr. Mazhar Osman Training and Research Hospital for Psychiatry, Neurology and Neurosurgery was obtained (date: November 5, 2019; protocol number: 374). The research was carried out in accordance with the Declaration of Helsinki.[20]

The study group consisted of schizophrenia patients who were followed up regularly in the institutions where the research was carried out and a healthy control group that was sociodemographically similar to these patients. It was planned to consecutively include 128 schizophrenia patients who attended follow-up studies between January 1, 2020, and November 1, 2020, at the relevant psychiatry clinics. Among these patients, 22 who did not meet the inclusion criteria or did not provide consent to participate in the study were excluded from the study group. The remaining 106 consecutive patients with schizophrenia (53 patients with schizophrenia in remission and 53 patients with treatment-resistant schizophrenia) were included in the study. A control group was formed with 53 healthy volunteers who were sociodemographically similar to the patients. Control participants were randomly selected from the same catchment area based on statistical records.

Each individual who accepted to participate in the research was given detailed information about the purpose and scope of the research, and it was stated that they could withdraw from the research at any stage without giving any reason. Written informed consent was obtained from those who agreed to participate in the study. For patients diagnosed with schizophrenia spectrum disorder according to Diagnostic and Statistical Manual-5 (DSM-5) diagnostic criteria, a data form including sociodemographic and clinical characteristics and questions of the scales used was filled. The questionnaire form applied to the healthy control group did not include questions about the scales.

Inclusion and Exclusion Criteria

For all individuals included in the study, inclusion criteria were being aged between 18 and 65 years, having graduated from primary school (at least), and having a body mass index value below 30. Exclusion criteria were being diagnosed with mental retardation or vision/hearing problems that could affect communication, history of alcohol and/or substance use disorders (alcohol or illicit drugs), being diagnosed with any other psychiatric diseases, history of chronic inflammatory systemic disease, and being diagnosed with any acute infectious diseases.

Specific inclusion criteria for the remission group were having received schizophrenia diagnosis according to DSM-5 criteria, not being hospitalized or receiving electroconvulsive therapy within the last 6 months, not having a history of episode or treatment change during the same period, and being in remission according to Positive and Negative Syndrome Scale (PANSS) score (remission is assessed using 8 items of the PANSS, all of which have to be scored with symptom severity of ≤3 points).

Specific inclusion criteria for the treatment-resistant schizophrenia group were being diagnosed with schizophrenia according to DSM-5 criteria, failure to respond to 2 antipsychotic treatments at a dose equivalent to at least 600 mg of chlorpromazine for at least 6 weeks (each antipsychotic), and having a Brief Psychiatric Rating Scale (BPRS) score of ≥45, a PANSS score of ≥75, a Clinical Global Impression Scale—Severity of Disease (CGI-S) score of ≥4, and a General Assessment of Functionality (GAF) score of ≤50.

Not being diagnosed with schizophrenia and not having a first- and/or second-degree relative with schizophrenia and/or similar psychiatric diagnoses were the specific inclusion criteria for healthy volunteers.

Complement Measurements

Blood samples were taken from the individuals in the research group after 12 hours of overnight fasting in serum separator tubes with a capacity of 5 mL. C3 and C4 serum values were measured after blood samples were centrifuged at 3000 rpm for an average of 10 minutes to obtain serum in the biochemistry laboratory. C3 and C4 levels were measured by nephelometry (Beckman Array Protein System, Newark, Del, USA).

Data Collection Tools

The data collection form used in the study included questions about the sociodemographic characteristics and medical history of individuals, the DSM-5 Structured Clinical Interview for DSM-5 (SCID-5), and the following scales: BPRS, PANSS, CGI-S, GAF, and Beck Cognitive Insight Scale (BCIS).

The Structured Clinical Interview for Diagnostic and Statistical Manual-5

The Turkish adaptation of SCID-5 carried out by Elbir et al[21,22] was used. The SCID-5 consists of 10 modules including psychotic symptoms, psychotic disorders, mood disorders, substance use disorders, anxiety disorders, obsessive compulsive disorder and related disorders, post-traumatic stress disorder, attention deficit and hyperactivity disorder, investigative questions for other disorders, and adjustment disorder.

The Brief Psychiatric Rating Scale

The BPRS, developed by Overall et al.[23,24] is used to measure the severity and change of psychotic and some depressive symptoms in schizophrenia and other psychotic disorders. Its Turkish validity and reliability study was performed by Soykan and colleagues. The scale is semi-structured and consists of 18 items evaluated between 0 and 6 points.

The Positive and Negative Syndrome Scale

The Turkish validity and reliability of the PANSS, initially developed by Kay et al.[25,26] was performed by Kostakoglu and colleagues. The PANSS consists of 30 items, including 7 items in the positive symptoms scale, 7 items in the negative symptoms scale, and 16 items in the general psychopathology scale. Total score (PANSS—total) and subdimension scores were recorded (PANSS—positive symptoms, PANSS—negative symptoms, PANSS—general psychopathology).

The Clinical Global Follow-up Scale

The CGI was used to evaluate the symptom severity, global improvement, and therapeutic response of the individuals. The scale, which enables the evaluation of patient functionality before and after treatment, was developed by Guy in 1976. There are 3 sub-dimensions in the scale that evaluate the severity of the disease, recovery, and drug side effects.[27]

The General Assessment of Functionality

The GAS scale was used to evaluate the psychological, social, and professional functionality of the individuals. The scale allows functionality to be rated at the time of measurement or in the past with a score ranging from 1 to 100. High scores on the scale indicate high functionality.[28]

The Beck Cognitive Insight Scale

The Turkish validity and reliability of the scale, initially developed by David et al.[29,30] was conducted by Aslan and colleagues. There are 3 components in the scale: adherence to treatment, being aware of the disease, and accurately recognizing psychotic experiences. There are 8 questions in the scale applied by the clinician, and the

highest total score that can be obtained is 18. A higher score indicates a higher level of insight.

Statistical Analysis

All analyses were subject to a significance threshold of $P < .05$ and were performed on SPSS version 20.0 (IBM SPSS Corp., Armonk, NY, USA). The Shapiro-Wilk test of normality was carried out on continuous variables to verify the fit of the data to a normal distribution. If it is a normal distribution, descriptive statistics were given as mean $\pm$ SD. If it is not a normal distribution, data were shown as median (1st quartile-3rd quartile) for continuous variables and as an absolute and relative frequency for categorical variables. Pearson chi-square test or Fisher's exact test was used to compare discrete variables (comparisons of schizophrenia patients according to treatment methods). Normally distributed variables were analyzed with the one-way analysis of variance test or independent samples t-test (comparison of C3 levels and age of disease onset between the groups due to the treatment response). Non-normally distributed variables were analyzed with the Mann-Whitney U-test or Kruskal-Wallis test depending on group counts being compared (comparison of descriptive characteristics (except for the age of disease onset), scale scores, and C4 levels of the schizophrenia patients due to the treatment response). Post hoc pairwise comparisons were performed with the Bonferroni post hoc testing. Spearman correlation coefficients were calculated to evaluate directional relationships between continuous variables (correlations between complement levels and scale scores). Multiple logistic regression analysis was performed to determine significant factors independently associated with treatment resistance.

RESULTS

Distribution of Patients with Schizophrenia According to Their Descriptive Characteristics

All patients included in the study groups, schizophrenia in remission (n=53), treatment-resistant schizophrenia (n=53), and healthy controls (n=53), were included in the analyses. Among the individuals, 105 (66.0%) were male, 54 (34.0%) were female, and the mean age was 40.11 $\pm$ 10.40 years. There was no significant difference between the 3 groups in terms of age ($P=.933$) and sex ($P=1.000$). The in-remission and treatment-resistant schizophrenia groups were similar in terms of age at disease onset ($P=.673$), age at treatment initiation ($P=.714$), duration of treatment ($P=.554$), duration with disease ($P=.752$), and number of hospitalizations ($P=.101$) (Table 1).

Distribution of Schizophrenia Patients According to Treatment Methods and Antipsychotic Medications

Polypharmacy (>2 antipsychotics) was the most commonly used therapeutic approach in both the in-remission and treatment-resistant schizophrenia groups. The frequencies

Table 1. Distribution of Patients with Schizophrenia According to Their Descriptive Characteristics

	In-Remission Schizophrenia (n=53)	Treatment-Resistant Schizophrenia (n=53)	P
Disease onset age	23.6 $\pm$ 6.5	24.6 $\pm$ 6.4	.673[a]
Age at onset of treatment	23 (20-30)	25 (21-30)	.714[b]
Treatment start time (years)	1 (0-2)	0 (0-2)	.554[b]
Disease duration (months)	170 (130-250)	165 (120-240)	.752[b]
Number of hospitalizations	2 (1-4)	3 (1-7)	.101[b]

Data are given as mean $\pm$ SD or median (first quartile-third quartile) for continuous variables according to normality of distribution.
[a]Independent samples t-test; [b]Mann-Whitney U-test.

of using long-acting injectable (LAI) antipsychotics ($P=.032$), oral paliperidone ($P=.028$), and oral quetiapine ($P=.045$) were significantly higher in the treatment-resistant schizophrenia group compared to the in-remission schizophrenia group. Other forms of treatment were similar between the 2 groups (Table 2).

Comparison of Scale Scores of the Treatment-Resistant Schizophrenia Patients with In-Remission Schizophrenia Patients

While the treatment-resistant schizophrenia patients had significantly higher BPRS ($P < .001$), PANSS—positive symptoms ($P < .001$), PANSS—negative symptoms ($P < .001$), PANSS—general psychopathology ($P < .001$), PANSS—total ($P < .001$), and CGI-S ($P < .001$) scores, in the treatment-resistant schizophrenia group, GAF ($P < .001$), and BCIS ($P < .001$) scores were significantly lower compared to those in remission (Table 3).

Comparison of C3 and C4 Levels Between the Groups due to the Treatment Response

C3 ($P=.001$) and C4 ($P=.001$) levels were found to be significantly higher in the treatment-resistant schizophrenia group compared to the in-remission schizophrenia group and healthy controls. The in-remission schizophrenia group and healthy controls had similar C3 and C4 levels (Table 4).

When post hoc pairwise comparisons were performed due to the Bonferroni post hoc testing, the C3 level was found to be statistically different in the treatment-resistant schizophrenia patient group from the patient group in remission ($P=.021$) and the healthy control group ($P=.001$). In contrast, no statistically significant difference was found between the patient group in remission and the healthy control group ($P=.897$). Again when post hoc pairwise comparisons for Kruskal-Wallis were performed, the C4 level of the treatment-resistant schizophrenia patient group was found to be statistically different from the patient group in remission ($P=.021$) and the healthy control group ($P < .001$). However, no statistically significant difference was found between the patient group in remission and the healthy control group ($P=.223$).

Table 2. Distribution of Schizophrenia Patients According to Treatment Methods

	In-Remission Schizophrenia (n=53)	Treatment-Resistant Schizophrenia (n=53)	Total (n=106)	P
Depot treatment	20 (37.73)	31 (58.49)	51 (48.11)	.032*
Electroconvulsive therapy	9 (16.98)	16 (30.18)	25 (23.58)	.109*
Amisulpride	3 (5.66)	6 (11.32)	9 (8.49)	.488†
Aripiprazole	9 (16.98)	8 (15.09)	17 (16.03)	.791*
Haloperidol	7 (13.20)	11 (20.75)	18 (16.98)	.301*
Clozapine	11 (20.75)	17 (32.07)	28 (26.41)	.186*
Olanzapine	11 (20.75)	9 (16.98)	20 (18.86)	.620*
Paliperidone	6 (11.32)	15 (28.30)	21 (19.81)	.028*
Risperidone	15 (28.30)	8 (15.09)	23 (21.69)	.099*
Sulpiride	1 (1.88)	0 (0.00)	1 (0.94)	1.000†
Zuclopenthixol	6 (11.32)	10 (18.86)	16 (15.09)	.278*
Quetiapine	9 (16.98)	18 (33.96)	27 (25.47)	.045*
Flupentixol	1 (1.88)	0 (0.00)	1 (0.94)	1.000†
Chlorpromazine	2 (3.77)	1 (1.88)	3 (2.83)	1.000†
Polypharmacy	28 (52.83)	37 (69.81)	65 (61.32)	.073*

Data are given as frequency (percentage) for categorical variables.
*Chi-square test; †Fisher's exact test.

Table 3. Comparison of Scale Scores of the Treatment-Resistant Schizophrenia Patients with In-Remission Schizophrenia Patients

	In-Remission Schizophrenia (n=53)	Treatment-Resistant Schizophrenia (n=53)	P*
BPRS	12 (10-15)	48 (47-51)	<.001
PANSS—positive symptoms	12 (7-13)	28 (25-29)	<.001
PANSS—negative symptoms	15 (14-17)	24 (21-28)	<.001
PANSS—general psychopathology	26 (24-28)	52 (50-55)	<.001
PANSS—total	53 (48-56)	105 (99-109)	<.001
GAF	65 (60-70)	40 (35-40)	<.001
CGI-S	3 (3-3)	5 (5-6)	<.001
BCIS	10 (6-12)	4 (3-5)	<.001

Data are given as median (first quartile–third quartile) for continuous variables according to normality of distribution.
*Mann-Whitney *U*-test.
BCIS, Beck Cognitive Insight Scale; BPRS, Brief Psychiatric Rating Scale; CGI-S, Clinical Global Impression Scale—Severity; GAF, General Assessment of Functionality; PANSS, Positive and Negative Syndrome Scale.

Table 4. Comparison of Complement 3 and Complement 4 Levels Between the Groups due to the Treatment Response

	In-Remission Schizophrenia (n=53)	Treatment-Resistant Schizophrenia (n=53)	Healthy Controls (n=53)	P	Pairwise Comparison P
C3	1.22 ± 0.21	1.33 ± 0.19	1.18 ± 0.18	.001*	IS-TS=**0.021**
					IS-HC=0.897
					TS-HC=**0.001**
C4	0.27 (0.22-0.31)	0.32 (0.28-0.37)	0.28 (0.3-0.30)	.001†	IS-TS=**0.021**
					IS-HC=0.223
					TS-HC < **0.001**

Data are given as mean ± SD or median (first quartile–third quartile) for continuous variables according to normality of distribution.
*One-way ANOVA test; †Kruskal-Wallis test.
ANOVA, analysis of variance; C3, complement 3; C4, complement 4; HC, healthy controls; IS, in-remission schizophrenia; TS, treatment-resistant schizophrenia.

Table 5. Correlations Between Complement Levels and Scale Scores with Respect to Groups and Overall

	In-Remission Schizophrenia (n=53)		Treatment-Resistant Schizophrenia (n=53)		Total	
	C3	C4	C3	C4	C3	C4
	r; P*	r; P*	r; P*	r; P*	r; P*	r; P*
BPRS	0.002; .986	0.064; .650	−0.235; .090	−0.105; .454	0.173; .076	**0.279; .004**
PANSS—positive symptoms	−0.063; .652	0.075; .593	−0.178; .202	−0.051; .716	0.167; .086	**0.290; .003**
PANSS—negative symptoms	−0.014; .918	−0.086; .539	−0.129; .359	−0.097; .490	**0.194; .046**	**0.238; .014**
PANSS—general psychopathology	0.184; .187	0.230; .097	0.206; .138	0.052; .709	**0.322; .001**	**0.352; <.001**
PANSS—total	0.037; .792	0.130; .355	−0.066; .637	−0.058; .679	**0.218; .025**	**0.297; .002**
GAF	−0.184; .187	−0.234; .092	0.185; .185	0.253; .067	**−0.239; .013**	**−0.286; .003**
CGI-S	0.171; .220	0.130; .352	−0.008; .956	0.034; .809	**0.274; .004**	**0.330; .001**
BCIS	−0.069; .623	−0.138; .325	0.232; .095	0.258; .062	−0.164; .092	**−0.203; .037**

*Spearman correlation analysis.
BCIS, Beck Cognitive Insight Scale; BPRS, Brief Psychiatric Rating Scale; C3, complement 3; C4, complement 4; CGI-S, Clinical Global Impression Scale—Severity; GAF, General Assessment of Functionality; PANSS: Positive and Negative Syndrome Scale.

Correlations Between Complement Levels and Scale Scores with Respect to Groups and Overall

No significant correlations were observed between scale scores and complement levels when evaluated within the 2 groups. However, the C3 levels of patients with schizophrenia (overall) were positively correlated with PANSS—negative symptoms (P=.046), PANSS—general psychopathology (P=.001), PANSS—total (P=.025), and CGI (P=.004) scores. A negative correlation was found between C3 level and GAF (P=.013). Additionally, C4 levels were found to be positively correlated with BPRS (P=.004), PANSS—positive symptoms (P=.003), PANSS—negative symptoms (P=.014), PANSS—general psychopathology ($P < .001$), PANSS—total (P=.002), and CGI (P=.001) scores, whereas C4 levels were negatively correlated with GAF (P=.003) and BCIS (P=.037) scores. All correlation coefficients showed weak directional relationships (Table 5).

Multiple Logistic Regression Analysis of Resistance to Treatment in Patients with Schizophrenia

In the multiple logistic regression analysis performed to elucidate factors independently associated with treatment resistance in patients with schizophrenia, C3 (P=.580), educational status (P=.140), employment status (P=.881), disease onset age (P=.595), and treatment onset age (P=.275) were found to be nonsignificant. It was determined that higher serum C4 was associated with an increased likelihood of treatment resistance [odds ratio (OR): 1.133, 95% CI: 1.012-1.268; P=.030], whereas a higher BCIS score was associated with decreased likelihood of treatment resistance (OR: 0.317, 95% CI: 0.191-0.526; $P < .001$) (Table 6).

DISCUSSION

It has been reported that individuals affected by schizophrenia demonstrate changes in the functional activities of complements in addition to alterations in blood levels and expression profiles of the classical, alternative, and lectin complement pathways. Since complements are important mediators of immune response, these changes are speculated to play a role in the pathogenesis of schizophrenia.[31,32] In this study, we evaluated complement levels in patients with remission and treatment-resistant schizophrenia. Taken together, our results demonstrate

Table 6. Multiple Logistic Regression Analysis of Resistance to Treatment in Patients with Schizophrenia

	B	SD	Wald	Exp(B) (95% CI)	P
Constant	−1.289	2.653	0.236	0.276	.627
C4	0.125	0.058	4.700	1.133 (1.012-1.268)	**.030**
C3	0.010	0.018	0.307	1.010 (0.975-1.046)	.580
Educational status	0.186	0.126	2.179	1.205 (0.941-1.543)	.140
BCIS	−1.149	0.258	19.779	0.317 (0.191-0.526)	**<.001**
Working status	−0.285	1.909	0.022	0.752 (0.018-31.730)	.881
Disease onset age	−0.051	0.096	0.282	0.950 (0.787-1.147)	.595
Treatment onset age	0.098	0.089	1.194	1.103 (0.925-1.314)	.275

Omnibust test: X^2: 88.089, $P < .001$, −2 log probability: 58.858, Nagelkerke R^2: 0.753, Hosmer and Lemeshov Test X^2: 5.685, P: .683, dependent variable: treatment resistance, no (0); yes (1).
C3, Complement 3; C4, complement 4; BCIS, Beck Cognitive Insight Scale.

that patients with treatment-resistant schizophrenia have significantly worse scale scores, and higher C3 and C4 levels compared to those with in-remission schizophrenia. Furthermore, the similar levels of C3 and C4 in healthy controls and in-remission patients lends credibility to the proposed influence of complements in schizophrenia pathophysiology.

Complement proteins play a critical role not only in the immune system but also in neurodevelopment. By inducing differentiation, maturation, and migration of neuronal progenitor cells with C3a and C5 neuroprotective effects, they can influence neurogenesis following ischemic injury, reduce apoptosis, and provide dose-dependent protection against neurotoxicity.[33] In addition, it has been reported in mice that astrocyte-mediated C3 accumulation is detected in the early stages of life in the synapses targeted for synaptic pruning during development.[34] In their study, Bilimoria and Stevens reported that C1q and C3 proteins mediate activity-dependent synaptic elimination in the developing rodent brain, preferentially marking less-active synapses for later removal by microglia.[35] It has been reported that abnormal activation of the complement pathway may be effective in the pathogenesis of schizophrenia by causing cortical gray matter loss.[36] Furthermore, these possible relationships are supported by the strong relationships between schizophrenia and the MHC gene region that contains complement system genes.[13]

In the current study, while in-remission schizophrenia patients and healthy controls were found to be similar in terms of complement levels, the C3 and C4 levels in treatment-resistant schizophrenia patients were significantly higher compared to the other groups. Previous studies on this topic are largely conflicting: some report C3 and C4 increase, others show a decrease, while some researchers have not found significant differences from controls in schizophrenia patients.[19,37-39] In the study of Sekar et al.[13] the association of schizophrenia with the MHC locus was attributed to the alleles of the C4 genes, which were suggested to contribute to schizophrenia in proportion to their tendency to increase the expression of C4A and C4B.[13] In the study by Hakobyan et al.[40] the mean values of the hemolytic activities of C1, C3, and C4 complement components in schizophrenia patients were found to be higher than controls. These relationships are not only relevant for development of schizophrenia; Laskaris et al[12] showed significantly higher C4 levels in patients with chronic schizophrenia and higher C3 and C4 levels in individuals at high risk for psychosis. Furthermore, Idonije et al[37] reported that C3c was higher in schizophrenia patients receiving antipsychotic treatment compared to newly diagnosed patients, providing further support for an association between complement levels and disease severity or progression. Mixed evidence has been presented in studies published in the literature on the dysregulation of the complement system in schizophrenia.

It was thought that the differences regarding disease duration, hospitalization need, and symptom severity, and the methodological differences in complement quantification (activity-based or concentration-based) could lead to different evidence in terms of outcomes. In addition to these, differences in treatment compliance, type of treatment, and the duration of treatment may have also caused the observed variations in results.

In the present study, a positive correlation was found between C3 and C4 levels and clinical characteristics, including symptom severity, which are in line with contemporary literature. In addition, it was found that higher C3 and C4 levels were associated with lower functionality. Although a large number of studies have reported on the effects of altered complement levels in schizophrenia, uncertainty remains regarding the trend and clinical significance of these alterations from the onset of schizophrenia and during the course of disease and its treatment. Few studies have evaluated the relationship between complement levels and clinical characteristics of schizophrenia patients. One such study by Li et al[38] reported that there was a negative correlation between C3 level and PANSS scores. In the study of Laskaris et al.[12] it was reported that the severity of positive and negative symptoms was higher in schizophrenia patients with high C4 and low C3. Also, Morera et al[41] reported that C3 and C4 levels were positively correlated with the PANSS—negative symptoms subdimension. Although circulatory levels were not measured, Melbourne and colleagues reported that C4A mRNA level was positively associated with psychotic symptomatology, particularly the presence and severity of delusions.[42] Longitudinal studies that can more clearly reveal the effect of complement level on the onset of the disease and symptoms in the following process will be beneficial in patients with schizophrenia.

Schizophrenia is a highly heterogeneous disorder and treatment resistance is encountered in approximately 1 out of every 3 patients. The varying degrees of therapeutic response to antipsychotics may have important implications for clinical practice.[43] The relationship between complement levels and clinical outcomes, and therefore their potential role as a therapeutic target, remains unclear. In our study, it was found that increased C4 was an independent predictor of treatment resistance in patients with schizophrenia; however, C3 was nonsignificant. Sellgren et al[44] reported that variants at the C4 locus associated with schizophrenia risk caused increased complement levels, which was suggested as a possible mechanism mediating the development of psychotic symptoms, and neuronal accumulation and synapse uptake. In the study by Mondelli et al.[45] it was reported that baseline C4 levels predicted clinical outcomes at 1-year follow-up in patients with first-stage psychosis. In a study analyzing factors associated with nonresponsiveness to antipsychotic treatment, Enache et al[46] found that complement levels, including C3 and C4, had no effect on

treatment response, while IL-8 increase was independently associated with nonresponse to treatment. In the study by Zai et al.[47] it was reported that tardive dyskinesia, a movement disorder that can develop in schizophrenia patients receiving long-term antipsychotic medication, was associated with C4 copy count. Furthermore, Li et al[48] suggested that minor alleles predicting higher C4A level were associated with a lower degree of improvement in psychotic symptoms after treatment.

Additionally, the comparison of treatment types in the in-remission and treatment-resistant groups showed that patients in the latter group had received, LAI antipsychotics, oral paliperidone, and oral quetiapine more frequently. Although this relationship may be a direct result of the need for advanced treatment among patients with resistance, there is value in briefly assessing the literature on this topic. One large systematic review reported that adherence to treatment, time until response, and duration of pre-treatment disease were associated with resistance.[49] The authors suggested that using LAI antipsychotics at the first psychotic episode could benefit patients by reducing relapse likelihood. This is supported by the fact that extended periods of untreated psychosis have been associated with treatment resistance,[50,51] whereas early treatment for psychosis appears to increase response and remission.[52]

This research has several limitations. First, as this research has a cross-sectional design, no assumptions about causality can be made. Another limitation is the relatively small sample size of the research and the lack of a community-based research plan. Although patients with symptoms that could indicate infection and individuals with diseases that could alter immunity-related parameters were excluded from the study, any condition other than schizophrenia that could change complement levels may have affected our results. Measurement of complement protein levels in peripheral blood instead of cerebrospinal fluid may have also affected our results. Because most complement proteins do not cross the blood–brain barrier, it is possible that concentrations of complement proteins in serum or plasma did not reflect complement activity in the brain. Evaluation of the level of other complements and inflammatory biomarkers within the scope of the study could have produced more comprehensive results. Another limitation is that patients with schizophrenia are highly heterogeneous in terms of the treatments they receive. We could not control for possible differential effects of drugs on complement levels. Lastly, the lack of Turkish validity and reliability studies of some of the scales we used in our research (CGI, GAF) may also be among the limitations. Despite these limitations, this study is remarkable for the fact that complement levels were measured among patients in remission and those with treatment-resistant schizophrenia and that the relationships between complement levels and a battery of scales were assessed.

Our findings show that C3 and C4 levels were higher in treatment-resistant schizophrenia patients compared to those in remission and also healthy controls. C3 and C4 levels were also correlated with symptom severity in patients with schizophrenia, albeit relationships were weak. Higher C4 levels and lower BCIS scores were found to increase the likelihood of treatment resistance in schizophrenia. In the light of the analyses, it is feasible to suggest that peripheral complement concentrations may be associated with the schizophrenia pathogenesis, and imbalances in complement levels may be associated with symptom severity and treatment resistance. In schizophrenia, serum C3 and C4 levels can be used as a potential biomarker to assess clinical symptoms, and serum C4 levels could be a marker to predict resistance to treatment. However, prospective longitudinal studies are needed to confirm our findings before definitive claims can be made regarding complement levels and schizophrenia symptom severity, and treatment resistance. Future clinical studies with anti-inflammatory drugs could investigate the connection between changes in serum C3 and C4 levels and symptom improvements of schizophrenia.

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