Association of Glial Cell-Line Derived Neurotrophic Factor and Nerve Growth Factor with Duration of Untreated Psychosis and Clinical Symptoms in Drug-Naive Schizophrenia

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ABSTRACT

Background: The neurodevelopmental hypothesis is one of the most-emphasized hypotheses in the etiology of schizophrenia. Nerve growth factor (NGF) and glial cell-line derived neurotropic factor (GDNF) are neurotrophic factors that provide growth, differentiation, and survival in nerve cells in the development process. In this study, we aimed to compare the GDNF and NGF levels of schizophrenia patients with healthy controls and to analyze the relationship between the Positive and Negative Syndrome Scale (PANSS) scores, serum GDNF and NGF levels and the duration of untreated psychosis (DUP) of the patients.
Methods: The study involved 45 patients with a diagnosis of schizophrenia, who had never used any antipsychotic drug, and 45 age- and sex-matched healthy participants. The participants filled a sociodemographic data form. The PANSS was applied to evaluate the clinical conditions. Before the initiation of the treatment, serum samples were collected from the patients.
Results: The difference between the GDNF and NGF levels of the patient group and control group was statistically significant. The serum GDNF and NGF levels in schizophrenia patients were lower than healthy controls. No correlation was found between the DUP and serum GDNF and NGF levels. There was a positive correlation between general psychopathology and negative scores of PANSS and the DUP of patients.
Conclusion: GDNF and NGF levels seem to be indicators of schizophrenia and its progress; nevertheless, we still do not have sufficient information about these neurotrophic factors. The results of our study indicate that the neurodevelopmental changes occurring at the early stages of the illness prominently affect the progress of disease, highlighting the importance of treatment in the early stages of disease.

INTRODUCTION

Schizophrenia is a mental disorder that shows signs and symptoms in almost all aspects of the mental state, begins in youth, and leads to significant disability. The etiology of schizophrenia has not been fully clarified yet. Schizophrenia has a heterogeneous etiopathogenesis. Many hypotheses have been put forward about this subject. Today, there is a consensus that schizophrenia is a disease caused by the combination of many factors. According to the generally accepted stress-diathesis assumption, brain development deteriorates due to genetic or environmental factors in the early stages of development—this creates a predisposition—and symptoms of schizophrenia develop when the person encounters a stressful environmental impact in later life.[1] The predisposing environmental factors can be both biological (such as infection) and psychological (such as a stressful life event, childhood abuse).[2,3]

One of the most-emphasized hypotheses in the etiology of schizophrenia is the neurodevelopmental hypothesis.[4] Based on an examination of the cognitive, positive, and negative symptoms of patients diagnosed with schizophrenia, together with imaging findings, the "neurodevelopmental theory" was proposed.[5] According to this assumption, schizophrenia is a disorder begins during the development of the brain. In the early stages of neuronal development,

the normal maturation process of the brain is disrupted by the effect of some pathological processes that affect brain maturation and neuron development. It has been stated that this effect may occur during cell proliferation, differentiation, cell migration, synaptic pruning, and programmed cell death. It is thought that disruptions in signal transmission and neuronal circuits, which may occur as a result of all these effects on the development of the nervous system, lead to the emergence of schizophrenia symptoms.[6] The neurodevelopmental hypothesis suggests that any disruptions that occur in neuronal migration, neuronal connections, and neural plasticity lead to structural abnormalities in specific brain regions, which play an important role in the development of schizophrenia.[7]

The presence of growth-related abnormalities (low birthweight, late maturation, large ventricles, atrophy, etc.) in many cases of schizophrenia supports the neurodevelopmental hypothesis.[8-11] Neuronal growth and development are mediated by growth factors synthesized by the glial cells.[12-14] In patients with schizophrenia, increased levels of S100B protein, an indicator of glial cell integrity, have been shown. This suggests that glial growth factors play a role in the pathogenesis of schizophrenia.[15,16]

Nerve growth factor (NGF) and glial cell-line derived neurotrophic factor (GDNF) are neurotrophic factors that provide growth, differentiation, and survival in nerve cells in the development process, and also have a role in continuity and plasticity in adult neurons.[17] Primary changes in the activities of these molecules lead to inappropriate changes in the cortical circuitry and synaptic transmission in the developing brain. GDNF is one of the most important neurotrophic factors in the mammalian brain.[18] The most important effect of GDNF is its contribution to support the survival of motor and dopaminergic neurons.[19] Therefore, GDNF may be potentially relevant to the neurodevelopmental hypothesis of schizophrenia. Studies published on GDNF levels in schizophrenia[20-23] have shown inconsistent results; 2 studies suggested that serum GDNF levels were associated with cognitive symptoms.[22,23] NGF is an important neurotrophic factor which has a likely role in the pathophysiology of several CNS disorders, including schizophrenia and depression.[24,25] NGF and its receptor are the essential mediators of synaptic and morphological plasticity, neuronal growth, survival, and differentiation, especially in the developing brain.[26,27] NGF also has a role in the regulation of the responsiveness of immune-competent cells.[28] A meta-analysis of studies on NGF levels in schizophrenia demonstrated that patients with schizophrenia are accompanied by decreased peripheral blood NGF levels, strengthening the clinical evidence of an abnormal neurotrophin profile in the patients with schizophrenia.[29] There is emerging evidence that dysfunctional NGF signaling may contribute to structural brain alterations in schizophrenia patients. Serum NGF levels have been found to be significantly correlated with smaller gray matter volume in the left prefrontal lobe and left midcingulate cortex in schizophrenia patients.[30] Studies have shown that atypical antipsychotics used in the treatment of schizophrenia increase NGF and GDNF levels.[31] It was observed that the patients treated with atypical antipsychotics had greater improvement in cognitive functions than those treated with typical antipsychotics. In addition, the NGF levels that increase with atypical antipsychotic treatment are associated with a decrease in negative symptoms.[32] Shao et al.[33] examined the GDNF release from the C6 glioma cells of rats by antipsychotic drugs (clozapine, quetiapine, and haloperidol), and found that these 3 antipsychotic drugs increased the GDNF release.[33] In recent years, there are more studies indicating that atypical antipsychotics increase the levels of neurotrophic factors.

Most of the studies on GDNF and NGF in schizophrenia patients reveal that serum GDNF and NGF levels are significantly decreased in schizophrenia patients compared to healthy controls.[34,35] However, since these studies were conducted with schizophrenia patients under treatment or patients with acute psychosis, the effect of the treatment could not be adequately excluded or the duration of untreated psychosis (DUP) not be evaluated. Our aim in this study is to compare the serum GDNF and NGF levels of schizophrenia patients, who have never received antipsychotic treatment, with a healthy control group and to analyze the relationship between the PANSS scores, GDNF, NGF levels and DUP. To our knowledge, this is the first study to examine GDNF and NGF levels in untreated schizophrenia patients and the relationship between these levels and the clinical features of the disease.

METHODS

Sample

The sample group of this analytical, case–control study consists of consecutive patients who presented to Ankara Numune Training and Research Hospital Psychiatry Clinic and were diagnosed with schizophrenia according to DSM-5 diagnostic criteria, and who had not used any antipsychotic

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MAIN POINTS

- The "neurodevelopmental model" is one of the most-emphasized hypotheses in the etiology of schizophrenia. Nerve growth factor (NGF) and glial cell-line derived neurotropic factor (GDNF) are neurotrophic factors that provide growth, differentiation, and survival in nerve cells in the development process.
- In this study, serum GDNF and NGF levels were found to be lower in the patient group compared to the healthy controls.
- In addition to the literature, we found that serum GDNF and NGF levels are significantly reduced in untreated schizophrenia patients. We also found that this decrease is not significantly correlated with the DUP of patients.

drugs earlier nor were currently using any. A total of 90 people, 45 of whom were schizophrenic patients and 45 age and sex-matched healthy controls, were included in the study. The patients, their relatives, and healthy volunteers were informed about the study and their written consent was obtained.

The recruitment criteria for patients were first-time diagnosis of schizophrenia (according to DSM-5 diagnostic criteria) and informed consent by the patient and a first-degree relative. The exclusion criteria for the study were intellectual disability, another axis-I illness, alcohol or substance use disorders (according to SCID-I), the presence of metabolic or endocrinologic illness, and using another medication for other reasons. The control group consisted of volunteers matched with schizophrenia patients in terms of age and gender and were physically and mentally healthy, with no history of schizophrenia or psychotic disorders in their first-degree relatives.

Data Collection and Evaluation Tools

Sociodemographic Information Form: It is a semi-structured form used to determine the sociodemographic characteristics of the patients and the control group participating in the study. After the psychiatric interview, the sociodemographic information form was filled out. This form includes questions on age, gender, marital status, educational status, occupation, duration of illness, family history of psychiatric illness, substance or drug use, and cigarette consumption.

Semi-Structured Clinical Interview for DSM-5 Axis-I Disorder (SCID-I): It is a structured clinical interview scale developed by Spitzer et al. for DSM-5 Axis-I diagnoses.

Positive and Negative Syndrome Scale (PANSS): Developed by Kay et al. (1987),[36] this scale is used to measure the level, distribution, and severity of the positive and negative symptoms of schizophrenia. The study of validity and reliability for the Turkish version of this test was conducted by Kostakoglu et al.[37] It is a scale evaluated by the interviewer. It includes 3 subscales and 30 items in total. These subscales are Positive symptoms, Negative symptoms, and General psychopathology.

Measurement of Serum GDNF Levels: Serum GDNF levels were measured by a commercial ELISA kit (Hangzhou Eastbiopharm Co. Ltd., Guangzhou, China. Cat.No: CK-E10166). The within-run CV (Coefficient of variability) % is ‹10, interstudy CV% is ‹12. Its sensitivity is 0.02 ng/mL and the measuring range is 0.05-20 ng/mL. The work was done in accordance with the test kit instructions.

Measurement of Serum NGF Levels: Serum NGF levels were measured by a commercial ELISA kit (Hangzhou Eastbiopharm Co. Ltd., Guangzhou, China. Cat. No: CK-E10126). Within-run CV% is ‹10, interstudy CV% is ‹12. Its sensitivity is 2.48 pg/mL and its measurement range is 5-1200 pg/mL. The work was done in accordance with the test kit instructions.

Procedure

Consecutive patients who applied to Ankara Numune Training and Research Hospital Psychiatry Outpatient Clinic, who had never used any antipsychotic medication before and were not using at the time of application were referred to the relevant physician. In the psychiatric interview with the patients, the appropriateness of the diagnosis of "Schizophrenia" in Axis-I according to the DSM-5 with SCID-I, and the fact that the symptoms continued for 6 months or more, were confirmed. A physical examination of the patients meeting the inclusion criteria was performed. An information form containing sociodemographic characteristics was filled out. The PANSS scale was applied to evaluate the clinical conditions of the patients. The clinical doctor planned the treatment, and there was no intervention in this treatment protocol. Serum samples to measure GDNF and NGF levels were obtained before the treatment was initiated.

Venous blood samples of the subjects participating in the study and the control group were taken into biochemistry tubes under identical conditions after an overnight fast, between 08.00 AM and 09.00 AM, and a 30-minute waiting period for the blood samples to clot was allowed. Subsequently, the samples were centrifuged at 3500 rpm for 10 minutes at room temperature. The serum portion of the samples was separated, and the serum samples were stored at −70°C until measurement.

Ethical Statement

This study was conducted in accordance with the Declaration of Helsinki, and the approval for the study was obtained from the Ethics Committee of Ankara Numune Training and Research Hospital (decision number: 20796219-E. Kurul-E-14-142, date: March 27, 2014). The patients and their relatives were informed about the study, and 2 signed informed consent forms were obtained from each patient and a relative. The participants could withdraw from the survey at any moment without providing any justification.

Data Analysis

All data obtained were coded numerically and evaluated with the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM SPSS Corp.; Armonk, NY, USA). The numerical variables were expressed as mean ± standard deviation for parametric data, mean + interquartile range (IQR) for nonparametric data, and the categorical variables as frequency and percentage. In the comparison of paired groups, the Student's t-test was used for parametric data, and the Mann–Whitney U-test was used for nonparametric data. The Kruskal-Wallis test was used to compare the values of more than 2 groups, and for the groups that were found to be significant according to the results of this analysis, the differences between groups were tested using Tukey's multiple comparison test. In addition, to understand the

relationship between variables, the Pearson correlation test was used for parametric data, and the Spearman correlation test was used for nonparametric data. The significance level was taken as $P < .05$. The effect size of the study was detected by using Glass's delta method and the power of study was calculated with the post hoc power calculator of G-Power for Mac OS X Version 3.1.9.2.

RESULTS

Sociodemographic Characteristics of the Schizophrenia Patients and the Healthy Controls

An analysis of participants' demographics showed that 22 (48.9%) participants of both the patient and control group were female and 23 (51.1%) were male. The average age of patients was calculated as 32.11 ± 10.22 and that of the control group as 31.53 ± 9.39 ($P=.781$). The average period of education received (in years) of the patients was determined as 8.29 ± 3.82, and that of the control group as 15.00 ± 11.49 ($P < .001$). Twenty-seven of the patients (60.0%) were single, 11 (24.4%) were married, 7 (15.6%) were divorced/widowed. In the control group, 20 (44.4%) were single, 23 (51.1%) were married, and 2 (4.4%) were divorced/widowed. It was determined that 6 of the patients (13.3%) had a job, 39 (86.7%) did not have a job, and in the control group, 36 (80.0%) of the participants had a job and 9 (20.0%) did not. The sociodemographic characteristics of both groups are given in Table 1.

Clinical Characteristics of the Schizophrenia Patients

The average age of onset was 27.56 ± 8.00, and the average duration of illness was 4.16 ± 5.39 years. Six of

Table 2. Clinical Features of the Patients

Clinical Features	Schizophrenia Group
Age of onset	27.56 ± 8.00
Average duration of illness (year)	4.16 ± 5.39
Family history of schizophrenia	
Yes (%)	6 (13.3)
No (%)	39 (86.7)
Smoking	
Yes (%)	18 (40)
No (%)	27 (60)
PANSS negative	30.07 ± 3.86
PANSS positive	28.64 ± 2.81
PANSS general psychopathology	50.76 ± 4.05
PANSS total	109.56 ± 8.90

the patients (13.3%) had a family history of schizophrenia. Eighteen (40%) of the patients were smokers. When the PANSS scores of the patients were evaluated, the average PANSS negative subscale score was determined as 30.07 ± 3.86, the mean PANSS positive subscale score as 28.64 ± 2.81, the mean PANSS general psychopathology subscale score as 50.76 ± 4.05, and the average PANSS total score as 109.56 ± 8.90 (Table 2).

GDNF and NGF Levels of the Patient and Control Groups

The median GDNF level (IQR-interquartile distance) of the patient group was 2.87 (2.06-4.20) ng/mL. The median GDNF level (IQR) of the control group was 4.71 (3.23-14.30) ng/mL. While the median NGF level (IQR) of the patient group was 145.63 (130.63-237.13) ng/mL, the median NGF level (IQR) of the control group was 217.82

Table 1. Sociodemographic Characteristics and Serum GDNF and NGF Levels of the Patients and Healthy Controls

Demographic Features	Schizophrenia Group	Healthy Controls	P	
Sex				
Male (%)	23 (51.1)	23 (51.1)		
Female (%)	22 (48.9)	22 (48.9)		
Age (mean)	32.11 ± 10.22	31.53 ± 9.59	.781	
Marital status				
Married (%)	11 (24.4)	23 (51.1)	.095	
Single (%)	27 (60)	20 (44.4)		
Divorced/widowed	7 (15.6)	2 (4.4)		
Work status				
Working	6 (13.3)	36 (80)	**<.001**	
Unemployed	39 (86.7)	9 (20)		
Education level (years-mean)	8.29 ± 3.21	15 ± 11.49	**<.001**	
	Schizophrenia Group	**Healthy Controls**	*z*	*P*
GDNF	2.87 (2.06-4.20) ng/mL	4.71 (3.23-14.30) ng/mL	−3.256	**.001****
NGF	145.63 (130.63-237.13) ng/mL	217.82 (153.75-936.89) ng/mL	−2.671	**.008****

*Student's *t*-test.
**Mann-Whitney *U*-test.
Bold characters: $P < .005$ - statistically significant.

Table 3. Relationship of Sex with GDNF, NGF Levels, and PANSS Scores

	Male	Female	z	P
GDNF	2.66 (2.09-3.51)	2.93 (1.88-5.92)	−0.363	.716
NGF	144.38(129.38-191.25)	158.75(131.25-279.08)	−0.693	.489
PANSS negative	30.22 ± 3.64	29.91 ± 4.15	−0.183	.855
PANSS positive	29.22 ± 2.21	28.05 ± 3.27	−1.532	.126
PANSS general psychopathology	50.91 ± 4.55	50.91 ± 4.55	−0.285	.776
PANSS total	110.39 ± 8.22	108.68 ± 9.68	−0.683	.495

Table 4. Relationship of Family History with GDNF, NGF Levels, and PANSS Scores

	Positive Family History (n=6)	Negative Family History (n=39)	z	P
GDNF	2.38 (0.42-3.25)	2.88 (2.09-4.57)	−1.302	.193
NGF	151.88 (114.26-200.99)	145.00 (129.38-250.50)	−0.267	.789
PANSS negative	30.17 ± 4.83	30.05 ± 3.76	−0.605	.545
PANSS positive	27.83 ± 2.04	28.77 ± 2.92	−0.034	.973
PANSS general psychopathology	51.17 ± 2.93	50.69 ± 4.22	−0.168	.867
PANSS total	109.17 ± 8.75	109.62 ± 9.04	−0.184	.854

*Mann–Whitney U-test.

(153.75-936.89) ng/mL. The difference between the GDNF and NGF levels of the patient group and the GDNF ($z =−3.256$, $P=.001$) and NGF levels of the control group was statistically significant ($z=−3.256$, $P=.001$; $z=−2.671$; $P=.008$) (Table 1). The study's effect size was detected as 0.61, and the power of the study was detected as 98% for GDNF levels, and 0.53 effect size, 93% power for NGF levels, with alpha values as 0.05.

Evaluation of Factors that may be Associated with GDNF and NGF Levels of the Patients Participating in the Study

When the participants were evaluated in terms of gender, no statistically significant difference was found between genders for both GDNF and NGF levels and PANSS scores (Table 3). The family history of the patients was examined and no statistically significant difference was found between the presence and absence of family history for both GDNF and NGF levels and PANSS scores (Table 4). There was no statistically significant difference in GDNF and NGF levels between smoking and non-smoking groups in the patient group. When the relationship between the age and duration of education of the patients and GDNF and NGF levels was examined, no correlation was found

with GDNF and NGF levels (Table 5). When the relationship between the disease onset age of the patients and GDNF ($r=0.188$, $P=.216$) and NGF ($r=0.083$, $P=.589$) levels was examined, no correlation was found. No correlation was found between GDNF and NGF levels when the patients were examined to determine whether there was a relationship between DUP of patients and GDNF ($r=0.202$, $P=.322$) and NGF ($r=−0.018$, $P=.931$) levels. When the relationship between the patients' PANSS scores and GDNF and NGF levels were examined, no correlation was found (Table 6). No correlation was found between GDNF ($P=.6$), NGF ($P=.727$) levels and DUP. There was no correlation between DUP and PANSS positive ($P=.813$), negative ($P=.454$) and general psychopathology ($P=.203$) scores. When the patients were categorized in accordance with the DUP, as 0-1 year, 1-5 years, and 5 years above, there was no significant difference in GDNF and NGF levels among the 3 groups ($P=.539$, $P=.139$). The statistical difference between the groups in terms of PANSS scores was detected in the negative, general psychopathology, and total scores (Table 7). When the group with a 0-1 year

Table 5. Relationship Between the Age and Duration of Education of the Patients and GDNF, NGF Levels

	GDNF	NGF
Age	$r=0.226$	$r=−0.055$*
	$P=.136$	$P=.718$
Duration of education	$r=−0.158$	$r=−0.056$*
	$P=.301$	$P=.715$

*Spearman Correlation Test.

Table 6. Relationship Between GDNF, NGF Levels and PANSS Scores

	GDNF	NGF
PANSS negative	$r=0.190$	$r=0.211$
	$P=.212$	$P=.163$
PANSS positive	$r=0.049$	$r=−0.003$
	$P=.749$	$P=.985$
PANSS general psychopathology	$r=0.104$	$r=0.117$
	$P=.495$	$P=.443$
PANSS total	$r=0.180$	$r=0.166$
	$P=.238$	$P=.277$

Table 7. Relationship Between Treatment-Free Period and GDNF Levels, NGF Levels, and PANSS Scores

	0-1 years (n=8)	1-5 years (n=23)	>5 years (n=14)	P
GDNF	2.03 ± 1.24	2.75 (2.01-4.57)	3.23 (2.47-12.63)	.539
NGF	137.37 ± 45.81	145.00 (129.38-250.50)	155.00 (127.19-771.36)	.139
PANSS negative	26.25 ± 1.49	31.39 ± 2.71	30.07 ± 4.97	**.005***
PANSS positive	28.00 ± 1.51	29.39 ± 3.06	27.79 ± 2.79	.331
PANSS general psychopathology	48.00 ± 2.20	51.43 ± 3.79	51.21 ± 4.77	**.046***
PANSS total	102.25 ± 4.06	112.26 ± 6.78	109.2 ± 11.64	**.023***

Bold characters: $P < .005$ - statistically significant.

DUP was compared with the group with 1-5 years, GDNF and NGF levels were lower in the 0-1 year group, but not statistically significant. A statistical difference between the 2 groups in terms of PANSS scores was detected in the negative ($z=-3.754$, $P < .001$), general psychopathology ($z=-2.708$, $P=.007$), and total ($z=-3.258$, $P=.001$) scores. There was no statistically significant difference in GDNF and NGF levels, and the PANSS scores when compared between the group with a 0-1 year DUP and the group with more than 5 years. There was no statistical difference in terms of GDNF, NGF levels, and PANSS scores when the group with 1-5 years and the group with over 5 years was compared. When the sociodemographic characteristics of the patients (age, marital status, education period, employment status) and clinical characteristics (age of onset of the disease, duration of the disease, smoking) were controlled, a moderate ($r=0.587$, $P=.021$) correlation was found between GDNF and PANSS negative scores, and a moderate correlation ($r=0.556$, $P=.031$) between NGF and PANSS negative scores.

DISCUSSION

It has been known for many years that schizophrenia is a chronic disease that causes social, cultural, and cognitive damage. In our study, in the schizophrenia group, compared to the control group, the duration of education, marriage and continuation of the marriage, and the working rates were significantly lower. Although it is stated in the literature that social and cognitive retardation reduces the levels of neurotrophic factors, it is controversial which one is the cause, and which one is the effect. In our study, low serum GDNF and NGF levels in the schizophrenia group may be associated with social, cognitive, and professional retardation.

In our study, serum GDNF and NGF levels were found to be lower in the patient group compared to the control group, and these differences were statistically significant. In a study conducted by Tunca et al.,[23] the serum BDNF and GDNF levels of 171 schizophrenia patients and 78 healthy controls were compared, and levels of both factors were found to be significantly lower in schizophrenia patients. In addition, low serum GDNF levels were found to be associated with a long duration of the disease.[25] In a meta-analysis including

22 case-control studies, it was reported that serum BDNF, NGF, and neurotrophin (NT) 4/5 levels were lower in schizophrenia patients with and without medication compared to the control group, and no relationship was found between neurotrophin levels, PANSS scores, and disease duration.[38] Xiong et al.,[34] with 30 untreated schizophrenia patients, 30 major depression patients, and 28 healthy controls, found that serum NGF and IL-2 levels of schizophrenia and depression patients were lower than the control group. They predicted that NGF and IL-2 could be screening tests for schizophrenia.[36] Niitsu et al.[39] compared 63 treated schizophrenia patients with 52 healthy control group members in terms of serum GDNF levels and found no significant difference.[39] A study conducted by Esen-Danaci et al.[40] investigated the BDNF levels of schizophrenia patients with depressive symptoms and compared them with major depressive disorder (MDD) patients. They found that the MDD group had significantly lower BDNF levels compared to schizophrenia patients with depressive disorder and put forward that this difference supports the hypothesis of distinct etiologies.[40] In some of the studies conducted on the GDNF gene in the literature, no strong relationship was found between schizophrenia and the GDNF gene.[41-43] Reasons for the different results may be due to antipsychotic use, the severity of the disease, the patient profile and treatment, and the patient's genetic background.

When the patient group was evaluated in terms of serum GDNF and NGF levels and gender, no significant difference was found. There are publications in the literature stating that neurotrophic factors are lower in women than in men.[44] These results may indicate that the presence of schizophrenia is more significant than gender on GDNF and NGF levels.

When the patient group was evaluated in terms of serum GDNF and NGF levels and the presence of schizophrenia in first-degree relatives, no significant difference was found between GDNF and NGF levels in groups with different family histories.

When the patient group was compared with regard to its serum GDNF and NGF levels in smokers and non-smokers, no statistically significant difference was found. An important part of the neuroprotective effect of nicotine is carried out by the regulation of neurotrophic factors. Nicotine is

a natural alkaloid. It has a significant stimulating effect on the central nervous system.[45] Different results have been obtained in studies examining the relationship of nicotine with neurotrophic factors. French et al.[46] found that the activation of nicotinic receptors in the hippocampus caused a temporary increase in the level of all neurotrophic factors.[46] Again, another study by French et al.[47] found that chronic nicotine usage lowers NGF levels in the hippocampus.[47] In our study, no relationship was found between serum GDNF and NGF levels and smoking.

Although the average age of disease onset of the patients participating in our study is compatible with the literature, the DUP is considerably longer than the periods in other studies conducted on the subject. This may be an indication that schizophrenia patients seek treatment later in our country due to cultural reasons and fear of stigmatization. There are studies in the literature show that cultural factors and value judgments change the perception of illness.[48,49] In our study, no significant correlation was found between the DUP and serum GDNF and NGF levels. This result could be interpreted to mean that serum GDNF and NGF levels decrease to a certain level at the beginning of the disease, but this decrease does not continue as the duration of the disease increases. To our knowledge, there is no study in the literature investigating the GDNF and NGF levels and the DUP in patients with schizophrenia who have never used antipsychotic drugs. Studies on BDNF, one of the most studied neurotrophic factors in schizophrenia patients, suggest conflicting results.[50-52]

When the sociodemographic and clinical characteristics of the patients included in our study were controlled, it was found that serum GDNF and NGF levels and PANSS Negative scores have a significant positive correlation. In other words, higher GDNF and NGF scores were found to be associated with more severe negative symptoms. Results in studies related to the subject in the literature vary. In the study by Tunca et al.,[23] no relationship was found between serum GDNF levels and PANSS scores.[25] Niitsu et al.[39] found a positive relationship between serum GDNF levels and the attention-assessing subscale of the Scale for the Assessment of Negative Symptoms in patients with schizophrenia. According to this study, high GDNF levels are correlated with attention deficiency in patients with schizophrenia.[39] Parikh et al.[32] found a negative correlation between NGF and PANSS negative scores in untreated acute psychosis patients (duration of illness <5 days).[34] In other words, negative symptoms are more severe in those with low NGF levels. These differences may be due to the different clinical conditions of the patients, whether they received treatment or not, the duration of the disease, or the ethnic variation in allele frequency due to GDNF and NGF gene polymorphisms.

In our study, when PANSS scores were compared in terms of time without treatment, it was found that PANSS negative, PANSS total, and PANSS general psychopathology scores increased significantly with the increase of patient's DUP. No statistically significant relationship was found between the PANSS positive scores and the DUP. This may be due to the fact that all of the patients we included in the study were in an active psychotic attack when the PANSS scales were filled out, and the positive symptoms were severe enough to require hospitalization. The increase in PANSS negative scores as the patients' DUP increases can be explained as an indication that schizophrenia is a chronic, destructive disease that causes regression in cognitive and social areas. These results are consistent with the literature.[53]

It is thought that reasons such as cultural values, religious beliefs, the acceptance of schizophrenia patients in the community more than in western societies, and the fear of stigmatization in our country delay the presenting of the patients to the hospital and prolong their duration without treatment. This situation created the opportunity to examine schizophrenia patients who had remained without treatment for a long time. When other studies in the literature were examined, it was found that either patients using treatment or first-attack psychosis patients were included, and in some studies, patients who did not use medication for a certain period (preceding 3 months) were considered as being without treatment.[54]

As for the limitations of our study, a higher sample size could increase the strength of the study. Although serum GDNF and NGF levels gave an indirect idea about cortical levels, the strength of the study could be increased if we could use a method that directly measured cortical GDNF and NGF levels.

In this study, we investigated the relationship between serum GDNF and NGF levels and the clinical features of previously untreated schizophrenia patients. The neurodevelopmental model of schizophrenia is one of the most discussed topics of recent years. GDNF and NGF are neurotrophic factors that play an important role in the neurodevelopmental model and the pathophysiology of schizophrenia. Our knowledge about these important neurotrophic factors is still not sufficient. In our study, in addition to the literature, we found that serum GDNF and NGF levels were significantly reduced in untreated schizophrenia patients, but this decrease was not significantly correlated with the DUP of patients. In light of the findings of our study, it is seen that neurodevelopmental changes in the initial years of schizophrenia significantly affect the course of the disease over the years, and this reveals the importance of treatment being started in the early stages of the disease. The information in the literature on the subject is quite contradictory and limited.

Future studies should be aimed at determining the changes in schizophrenia patients who remain without treatment and how these changes affect the disease process. This will also further help us understand the role of factors affecting the etiology of schizophrenia.

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