Can the Imbalance between Neurotrophic and Apoptotic Proteins Be the “Beware the Ides of March” for Unaffected Relatives of Schizophrenia Patients?

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Received: 16 November 2021 / Accepted: 22 September 2022 / Published online: 3 October 2022 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Schizophrenia (SZ) is a mental disorder with a strong genetic basis as well as epigenetic aspects. Siblings of patients with SZ can share certain endophenotypes with the patients, suggesting that siblings may be important for distinguishing between trait and state markers. In the current study, we aimed to characterize the balance between pro-BDNF/mature BDNF and its receptors p75NTR/TrkB, which are tPA-BDNF pathways proteins and are thought to play a role in synaptic pruning, as a possible endophenotype of schizophrenia. Forty drug-naïve patients with first-episode psychosis (FEP) matched for age, gender, and level of education, 40 unaffected siblings (UAS) of patients with FEP, and 67 healthy controls (HC) were included in the study. Blood samples were collected from all participants to determine BDNF, pro-BDNF, TrkB and p75NTR, PAI1, tPA, ACTH, and cortisol levels. We showed that levels of proteins of the tPA-BDNF pathway as well as the pro-BDNF/m-BDNF and p75NTR/TrkB ratios could successfully differentiate FEP and their siblings from the HCs by using ROC analysis. Plasma levels of m-BDNF were found to be the lowest in the healthy siblings and highest in the HCs with statistically significant differences between all 3 groups. The plasma level of pro-BDNF in the HC group was similar to the FEP patients, the same in the healthy siblings of the FEP patients. Our data support the hypothesis that imbalance between neurotrophic and apoptotic proteins might occur in SZ and this imbalance could be an endophenotype of the disease.

Keywords Apoptosis · BDNF · Endophenotype · Neurotrophin · Schizophrenia · Synaptic pruning

Introduction
Schizophrenia (SZ) is a progressive chronic mental disorder that affects 1% of the society and occurs primarily in late adolescence and young adulthood [1]. Although genome-wide association studies (GWAS) suggest that several thousand independent alleles of small effect confer susceptibility to SZ, both genetic and environmental factors may play a role in its pathogenesis [2]. To explain the pathogenesis of SZ, several hypotheses have been put forward including stress-diathesis, neurodevelopment, neurodegeneration, and neurotransmitter containing dopamine and glutamate hypothesis, as well as synaptic pruning [3].

Synaptic pruning is the selective elimination of synapses during synapse formation and refinement [4, 5]. Recent evidence suggests that synaptic pruning occurs in the postnatal period in early childhood and in young adulthood [6]. In vivo animal studies have shown that synaptic pruning occurs via apoptosis through a cooperation between microglia and the complement system; however, direct evidence for synaptic pruning in humans is currently lacking [7, 8]. Nonetheless, certain neuroimaging findings hint at its presence in humans. Longitudinal MRI studies in children showed that while the volume of white matter increased in early childhood, the volume of gray matter decreased during
adolescence [9], suggesting the occurrence of synaptic pruning. Although this synaptic loss during adolescence is physiological, excessive loss has been implicated in the pathogenesis of SZ. A greater reduction of gray matter volume in patients with SZ has been observed in neuroimaging studies [10]. Longitudinal studies with follow-up of subjects at high risk for psychosis and unaffected first-degree relatives of patients with SZ have shown that this decrease may be progressive [11, 12].

Brain-derived neurotrophic factor (BDNF) is a major neurotrophic factor that is synthesized from its precursor pro-BDNF in a pathway in which tissue plasminogen activator (tPA)/plasmin and cortisol are integral components [13]. By binding to its receptor tropomysin receptor kinase B (TrkB), BDNF is known to be essential for survival, differentiation, outgrowth, and synaptic plasticity of neurons, along with synthesis, metabolism, and release of neurotransmitters [14, 15, 16]. In addition, BDNF has other neuroprotective effects such as anti-apoptotic antioxidant activity, as well as suppression of autophagy. It is thought to have this effect especially via the induction of sonic hedgehog (SHH) via erythropoietin or the anti-apoptotic protein Bel-2 via the cGMP pathway [17]. Pro-BDNF, the precursor of BDNF that was previously considered to be an inactive protein, has recently been shown to act as a ligand for the neurotrophin receptor p75NTR (p75NTR) and plays important roles in several physiological functions such as neuronal death, spine retraction, and hippocampal long-term depression [13]. Thus, there may be a critical balance between the ligand-receptor complex of pro-BDNF/p75NTR and mature (m)-BDNF/TrkB [18]. Any potential imbalance in the levels of these proteins can play a role in the pathogenesis of neuropsychiatric disorders by enhancing synaptic pruning. We have recently reported an imbalance between pro-BDNF/m-BDNF and p75NTR/TrkB in patients with psychosis [19].

With the availability of genome sequencing, many studies on SZ have focused on disease risk of unaffected first-degree relatives of SZ patients. It was shown that the disease risk was eight fold for first-degree relatives of a single proband with SZ; this risk increased to 11-fold for first-degree relatives with two SZ probands [2]. Therefore, it is increasingly important to determine the disease risk of these groups early with the use of methods like endophenotypes.

Endophenotypes have recently gained importance, particularly for diseases such as SZ for which the pathogenesis remains unclear. Endophenotypes are independent of the state, are associated with the disease, and are seen in some unaffected relatives of individuals with disorders. They should be evaluated experimentally and cannot be visually distinguished [20].

**Aim of the Study**

The current study was designed to examine the hypothesis that an imbalance between neurotrophic and apoptotic proteins such as pro-BDNF/m-BDNF and p75NTR/TrkB can be used as endophenotypes for SZ.

**Material and Methods**

**Selection of Patients and Controls**

This study was conducted within the scope of a research project aiming to examine the tPA-BDNF pathway in patients with first-episode psychosis (FEP). A total of 65 patients with FEP were included in the project. Of these, 25 FEP patients were excluded from the current study because they did not have a sibling/unaffected sibling. Therefore, 40 drug-naïve FEP patients diagnosed according to SCID-5 were included in the study. Forty unaffected siblings (UAS) of FEP patients who did not have any history of psychiatric diseases were selected from among the brothers and sisters that were matched for age, gender, and level of education. Consent to participate in the study was obtained from both FEP patients and their siblings within the same age range. These patients were observed by an experienced psychiatrist over a period of 4–6 weeks. In addition, 67 healthy controls (HC) who were matched with the patients for age, gender, and marital status, with no previous psychiatric complaints, no psychiatric disorders identified in the psychiatric interview, as well as a negative family history for SZ and other psychotic disorders, were included in the study. HCs were selected from individuals who applied to the polyclinic for administrative procedures or from hospital staff who were routinely examined within the scope of occupational health and safety laws.

According to exclusion criteria, (1) patients, siblings, and HCs who were younger than 18 years of age or older than 40 years of age; (2) had less than 8 years of education; (3) were diagnosed with mental impairment or mental retardation; (4) had vision and/or hearing problems that could negatively affect communication; (5) had systemic and/or neurological diseases (hypertension; diabetes mellitus; infection; dementia; epilepsy; Parkinson’s disease; cardiovascular, renal, urological, hepatic, pulmonary, genetic, and endocrine diseases; or nutritional disorders); (6) had body mass index (BMI) lower than 18 or higher than 25; (7) had intoxications, surgery, and other organic disorders; (8) used nonpsychiatric drugs; (9) had a family history of psychiatric diseases (for HC); (10) had head trauma in the last 1 year; or (11) had a history of regular alcohol and/or substance abuse in the last 6 months were excluded from the study.

In addition, FEP patients with comorbid psychiatric diseases were not included in the study. A urine test was performed on all subjects to exclude current substance abuse. The minimum
sample size was calculated to be at least 120 participants (40 FEP + 40 UAS + 40 HC), by evaluating the effect size as 0.3, α-err as 0.05, and power as 0.80 with G Power of 3.1.9.2.

Study Procedure

FEP patients, UAS, and HC who met the inclusion criteria of the study were directed to the research team by their physicians. After the FEP patients, the UAS and HC were informed about the study; written informed consent was obtained from those who agreed to participate. Later, sociodemographic and clinical data forms were filled by all participants. Blood was collected in the morning after 10 h of fasting for both patient and control groups. Mature BDNF, pro-BDNF, TrkB, p75NTR, plasminogen activator inhibitor-1 (PAI-1), tPA, adrenocorticotropic hormone (ACTH), and cortisol levels were determined in both patient and control groups.

Ethical Approval

Prior to starting the study, each participant received an informed consent form stating the details of the research, and only those participants who consented to volunteer approved this form. All participants provided their written informed consent. Ethical approval for the study was obtained from an Ethical Committee of (University of Health Sciences Turkey) (IRB Date/Number; 17.09.2021/29–24). Financial support for the current research was provided by the University of (University of Health Sciences Turkey) Research Projects Unit (Date/Number; 07.03.2019/020).

Data Collection Tools

1) Sociodemographic and Clinical Data Forms: The sociodemographic data form included questions on characteristics such as age, gender, level of education, marital status, and level of income. The clinical data form included questions on the duration of illness, height, and weight. Both forms were filled by the patient and control groups.

2) Positive and Negative Syndrome Scale (PANSS): PANSS was used to assess the severity of disease in FEP patients. The scale, which was developed by Kay et al., is a semi-structured interview scale that consists of 30 components and a severity evaluation of 7 points. It consists of 3 fundamental sub-groups; 7 out of 30 components are for positive symptoms and 7 for negative symptoms, and 16 comprise a general psychopathology scale (Kay et al. 1987).

Blood Sample Collection

Following an overnight fast, venous blood samples (5 mL) were collected between 07:00 and 09:00 am in coagulant tubes with gel. The blood samples were left to clot at room temperature for 2 h and then centrifuged at 3000 rpm at 4 °C for 20 min to obtain the serum. The samples were separated into 0.5-mL aliquots and stored at −80 °C until use.

Protein Assays

Serum cortisol and ACTH levels were measured immediately after collection of the samples. Serum ACTH was measured using a two-site chemiluminescent immunometric assay with a commercially available kit (Immulel 2000; Siemens Healthcare Diagnostics, Germany). Serum cortisol was measured using a chemiluminescent Beckman Coulter DxI 800 immunoassay system (Beckman Coulter Inc., Brea, CA, USA). The total imprecision for these kits is reported to be <8.0%. Serum protein concentrations of mature BDNF, pro-BDNF, p75NTR, TrkB, tPA, and PAI-1 were measured by solid-phase enzyme-linked immunosorbent assay (ELISA) using commercial kits. The ELISA kits for BDNF (Catalog no: E1302Hu), pro-BDNF (E4070Hu), TrkB (E6633Hu), p75NTR (E4369Hu), tPA (E3707Hu), and PAI1 (E1159Hu) were purchased from BT Laboratory, China, and used according to the manufacturer’s instructions. In order to minimize assay variance, the protein concentrations of all samples were measured on the same day. All experiments were performed in duplicate. For each assay, intra-assay coefficient of variance (CV) was less than 8%, and inter-assay CV was less than 10%. No significant cross-reactivity or interference was observed.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 16.0 for Windows software package (SPSS Inc., Chicago, IL, US) was used for the statistical analysis. Descriptive statistical methods (frequency, percentage, mean, and standard deviation) were used for evaluating the study data. The variables were examined for normal distribution using visual and analytical tests. Chi-square and Fisher’s exact tests were used to compare categorical data of independent groups. The Kruskal–Wallis test and one-way ANOVA test were used to compare numerical data such as concentrations of mature BDNF, pro-BDNF, p75NTR, TrkB, tPA, and PAI-1 of independent group. For one-way ANOVA test, homogeneity was evaluated with Levene’s statistic test, and Tukey’s test was applied if the data showed homogeneous distribution. In the pairwise comparison of the data with significant differences in the Kruskal–Wallis test, the Bonferroni correction was applied, and p value was taken as 0.017. The potential of using the relevant biochemical parameters in distinguishing FEP/their UAS and HC was analyzed with
receiver operating characteristic (ROC) curves, \( p < 0.05 \) was accepted as the level of significance.

**Results**

**Sociodemographic Variables**

A comparison of the sociodemographic data of the groups included in the current study is shown in Table 1. Two of the characteristics were found to differ significantly between the groups. The proportion of participants actively working was found to be lower in the FEP patient group compared to that in the other two groups. The proportion of participants living alone was found to be higher in the HC group than that in the other groups. Apart from this, no significant difference was found between the two groups in terms of age, gender, marital status, education level, and birth-related data. The mean ± standard deviation (SD), min, and max values of all PANSS scores of the FEP group were as follows: \( \text{PANSS}_{\text{total}}: \text{mean} \pm \text{SD} 107.39 \pm 28.9, \text{min}: 70, \text{and max: 178} \); \( \text{PANSS}_{\text{positive}}: \text{mean} \pm \text{SD} 30.46 \pm 6.6, \text{min}: 21, \text{and max: 46} \); \( \text{PANSS}_{\text{negative}}: \text{mean} \pm \text{SD} 27.65 \pm 8.43, \text{min}: 12, \text{and max: 48} \); \( \text{PANSS}_{\text{general}}: \text{mean} \pm \text{SD} 48.25 \pm 17.78, \text{min}: 21, \text{and max: 85} \).

### Table 1 Comparison of sociodemographic and clinical data of patients with first-episode psychosis (FEP)/unaffected sibling of patients, and healthy control groups (mean ±SD (median))

|                          | Healthy controls (n=67) | Unaffected sibling of FEP (n=40) | FEP (n=40) | df  | \( p \) |
|--------------------------|-------------------------|----------------------------------|------------|-----|--------|
| **Age (years)**         | 26.6 ±7.7 (25.0)        | 27.1 ±7.8 (26.0)                | 26.5 ±6.04 (25.0) | 2   | 0.871  |
| **Gender (%)**          |                         |                                  |            |     |        |
| Female: 24 (35.8%)      | Male: 23 (57.5%)        | Male: 17 (42.5%)                | Male: 28 (70%)  | 2   | 0.507  |
| Male: 43 (64.2%)        | Female: 17 (42.5%)      | Female: 12 (30%)                | Female: 28 (70%) | 2   |        |
| **Education level (%)** |                         |                                  |            |     |        |
| College: 34 (53.1%)     | High school and below: 30 (46.9%) | High school and below: 21 (61.8%) | High school and below: 21 (60% | 2   | 0.267  |
| College: 14 (40%)       | College: 13 (38.2%)     | College: 14 (40%)               | College: 14 (40%) |     |        |
| **Marital status (%)**  |                         |                                  |            |     |        |
| Married: 25 (39.1%)     | Married: 17 (48.6%)     | Married: 9 (23.7%)              | Married: 9 (23.7%) | 2   | 0.082  |
| Other: 39 (60.9%)       | Other: 18 (51.4%)       | Other: 29 (76.3%)               | Other: 29 (76.3%) | 2   |        |
| **Occupational status (%)** |                    |                                  |            |     |        |
| Employed: 41 (64.1%)    | Employed: 21 (60%)      | Employed: 13 (34.2%)           | Employed: 13 (34.2%) | 2   | 0.011* |
| Unemployed: 23 (35.9%)  | Unemployed: 14 (40%)    | Unemployed: 25 (65.8%)         | Unemployed: 25 (65.8%) | 2   |        |
| **Relationship status (%)** |                    |                                  |            |     |        |
| Single: 20 (32.3%)      | Single: 0 (%)           | Single: 0 (%)                   | Single: 0 (%) | 2   | <0.001*|
| Other: 42 (67.7%)       | Other: 35 (100%)        | Other: 38 (100%)                | Other: 38 (100%) | 2   |        |
| **Delivery method (%)** |                         |                                  |            |     |        |
| Vaginal: 54 (91.5%)     | Vaginal: 33 (94.3%)     | Vaginal: 32 (88.9%)            | Vaginal: 32 (88.9%) | 2   | 0.716  |
| C/S: 5 (8.5%)           | C/S: 2 (5.7%)           | C/S: 4 (11.1%)                  | C/S: 4 (11.1%) | 2   |        |
| **Birth details (%)**   |                         |                                  |            |     |        |
| Term: 58 (98.3%)        | Term: 34 (97.1%)        | Term: 35 (97.2%)                | Term: 35 (97.2%) | 2   | 0.914  |
| Early or late birth: 1 (1.7%) | Early or late birth: 1 (2.9%) | Early or late birth: 1 (2.9%) | Early or late birth: 1 (2.9%) | 2   |        |
| **Disease during pregnancy (%)** |                   |                                  |            |     |        |
| Yes: 3 (5.1%)           | Yes: 2 (5.7%)           | Yes: 1 (2.9%)                   | Yes: 1 (2.9%) | 2   | 0.832  |
| **Smoking history (%)** |                         |                                  |            |     |        |
| Yes: 18 (28.6%)         | Yes: 14 (41.2%)         | Yes: 12 (37.5%)                 | Yes: 12 (37.5%) | 2   | 0.411  |

* \( p < 0.05 \), chi-square test, Fischer’s exact test, Mann–Whitney U-test, and Student’s t-test were used for statistical analyses. C/S: Delivery by cesarean section. In the pairwise comparison of the data with significant differences in chi-square test, the Bonferroni correction was applied, and \( p \) value was taken as 0.017

* \( p < 0.017 \) (compared with healthy control group)

### Comparison of the Serum Level of Biochemical Markers Related to the tPA-BDNF Pathway

A comparison of the biochemical markers between the three groups is shown in Table 2. The plasma level of m-BDNF was found to be the lowest in the UAS group (mean (M) = 2.82, SD = 1.84) and highest in the HC group (M = 7.61, SD = 3.50) with statistically significant differences identified between all three groups (\( p < 0.001 \)). The plasma pro-BDNF level of the HC group (M = 0.910, SD = 0.569) was similar to that of the FEP patients (M = 0.930, SD = 0.755, \( p = 0.877 \)). The same in the UAS group was lower (M = 0.539, SD = 0.664) than that in the FEP group (z = -2.569, \( p = 0.010 \)). Significant differences were identified in serum levels of most of the proteins related to the tPA-BDNF pathway between the three groups.

### Relationship between the Serum Level of Biochemical Markers Related to the tPA-BDNF Pathway

A statistical evaluation of the relationship between the biochemical markers is shown in Table 3. A statistically significant strong positive correlation was found between the levels of m-BDNF and pro-BDNF (\( r = 0.77 \)), p75NTR (\( r = 0.67 \)), TrkB (\( r = 0.92 \)), PAI-1 (\( r = 0.82 \)), and tPA (\( r = 0.72 \)). Similarly, a significant

\( p < 0.05 \), chi-square test, Fischer’s exact test, Mann–Whitney U-test, and Student’s t-test were used for statistical analyses. C/S: Delivery by cesarean section. In the pairwise comparison of the data with significant differences in chi-square test, the Bonferroni correction was applied, and \( p \) value was taken as 0.017

\( p < 0.017 \) (compared with healthy control group)

\( *p < 0.05 \), chi-square test, Fischer’s exact test, Mann–Whitney U-test, and Student’s t-test were used for statistical analyses. C/S: Delivery by cesarean section. In the pairwise comparison of the data with significant differences in chi-square test, the Bonferroni correction was applied, and \( p \) value was taken as 0.017

\( *p < 0.017 \) (compared with healthy control group)
strong positive correlation was found between the levels of pro-BDNF and p75NTR ($r = 0.93$), TrkB ($r = 0.85$), PAI-1 ($r = 0.88$), and tPA ($r = 0.88$). A negative correlation was identified between m-BDNF and cortisol levels ($r = -0.22$), but no significant correlation was found between pro-BDNF and cortisol.

### Identification of an Individual Protein to Differentiate FEP Patients and UAS from the HC Group

We analyzed the differential power of individual levels of m-BDNF, pro-BDNF, p75NTR, TrkB, and PAI-1 as well as the ratio of pro-BDNF/m-BDNF and p75NTR/TrkB by ROC analysis. Figure 1 presents the ROC curves for the serum level of these proteins for all three groups under study. We observed that individual levels of several of the proteins such as m-BDNF, pro-BDNF, p75NTR, TrkB, PAI-1, pro-BDNF/m-BDNF, and p75NTR/TrkB could help differentiate FEP and their UAS from HCs. For one-way ANOVA test, homogeneity was checked with Levene’s statistic test, and Tukey’s test was applied if it showed homogeneous distribution according to homogeneity status. In the pairwise comparison of the data with significant differences in the Kruskal–Wallis test, the Bonferroni correction was applied, and $p$ value was taken as 0.017

For one-way ANOVA test, homogeneity was checked with Levene’s statistic test, and Tukey’s test was applied if it showed homogeneous distribution according to homogeneity status. In the pairwise comparison of the data with significant differences in the Kruskal–Wallis test, the Bonferroni correction was applied, and $p$ value was taken as 0.017.

### Discussion

In the present study, plasma levels of proteins in the tPA-BDNF pathway, cortisol and ACTH of drug-naïve FEP patients, UAS of these patients, and HC were compared for possible endophenotypes.

### Table 2 Comparison of biochemical values of patients with first-episode psychosis/healthy sibling of patients, and healthy control groups (mean/median ± SD/SE)

|                  | Healthy controls (n=67) | Healthy sibling of FEP (n=40) | FEP (n=40) | df | p     |
|------------------|-------------------------|-------------------------------|------------|----|-------|
| m-BDNF†          | 7.61 ± 3.50(6.8)        | 2.82 ± 1.84(2.31)             | 4.84 ± 3.56(3.77)   | 2  | <0.001*   |
| Pro-BDNF†        | 0.910 ± 0.569(0.918)    | 0.539 ± 0.664(0.264)          | 0.930 ± 0.755(0.65) | 2  | 0.001*    |
| p75NTR‡          | 44.6 ± 45.3/4.55(34.2)  | 22.7 ± 40.5/6.4(6.12)         | 46.62 ± 50.3/7.9(31.4) | 2  | 0.006*    |
| TrkB‡            | 60.1 ± 36.8/4.56(68)    | 16.5 ± 26.8/4.2(7.42)         | 35.43 ± 35.8/5.6(22.5) | 2  | <0.001*    |
| PAI-1†           | 17.1 ± 9.91(20.9)       | 5.89 ± 6.68(3.42)             | 11.9 ± 9.46(9.14)   | 2  | <0.001*    |
| tPA†             | 50.1 ± 33.2(61.7)       | 24.8 ± 26.6(16.57)            | 41.18 ± 32.9(33.8)  | 2  | 0.003*    |
| ACTH‡            | 19.6 ± 16.1(15.5)       | 26.7 ± 11.9(23.6)             | 57.02 ± 52.16(44)   | 2  | <0.001*    |
| Cortisol†        | 8.62 ± 3.95             | 10.3 ± 3.42                   | 14.5 ± 5.3         | 2  | <0.001*    |
| pro-BDNF/m-BDNF† | 0.60 ± 0.53(0.47)       | 1.27 ± 0.94(1.38)             | 1.41 ± 0.91(1.54)   | 2  | <0.001*    |
| p75NTR/TrkB†     | 0.115 ± 0.46(0.109)     | 0.177 ± 0.098(0.180)          | 0.193 ± 0.086(0.188) | 2  | <0.001*    |

$p < 0.05$, FEP, first-episode psychosis; m-BDNF, mature brain–derived neurotrophic factor; pro-BDNF, precursor brain–derived neurotrophic factor; NTR, neurotrophic receptor; TrkB, tyrosine kinase beta; PAI-1, plasminogen activator inhibitor; tPA, tissue plasminogen activator; ACTH, adrenocorticotropic hormone

† One-way ANOVA test was used; ‡: the Kruskal–Wallis test was used

For one-way ANOVA test, homogeneity was checked with Levene’s statistic test, and Tukey’s test was applied if it showed homogeneous distribution according to homogeneity status. In the pairwise comparison of the data with significant differences in the Kruskal–Wallis test, the Bonferroni correction was applied, and $p$ value was taken as 0.017.

*a $p < 0.05$ or $p < 0.017$ (compared with healthy control group)

*b $p < 0.05$ or $p < 0.017$ (compared with healthy sibling of FEP group)

For one-way ANOVA test, homogeneity was checked with Levene’s statistic test, and Tukey’s test was applied if it showed homogeneous distribution according to homogeneity status. In the pairwise comparison of the data with significant differences in the Kruskal–Wallis test, the Bonferroni correction was applied, and $p$ value was taken as 0.017.

$a p < 0.05$ or $p < 0.017$ (compared with healthy control group)

*b $p < 0.05$ or $p < 0.017$ (compared with healthy sibling of FEP group)
The serum BDNF levels were found to be lower in the UAS than those in the FEP group; this level was lower in both FEP patients and their UAS compared to that in the HC. Since the discovery that BDNF can cross the blood–brain barrier, several studies (some on SZ) have evaluated the blood levels of BDNF [21]. Although most of SZ studies showed a decrease in BDNF levels in chronic SZ and FEP patients, some studies reported an increase, while others reported a lack of significant difference [22]. In the current study, no significant difference in serum pro-BDNF levels was found between the FEP group and HC, while the levels were significantly lower in the UAS compared to those in FEP and HC. Similarly, studies investigating the relationship between pro-BDNF and SZ reported a decrease in pro-BDNF values in SZ patients, while others reported no difference [23].

BDNF is synthesized in neurons via the formation of several precursor forms such as pre-pro-BDNF and pro-BDNF; the latter acts as a ligand for the TrkB receptor (Fig. 2a) [24]. BDNF plays an important role in synaptic plasticity, neuronal differentiation, dendritic growth, and branching by activating the PI3K/Akt/mTOR pathway [25]. BDNF can also contribute to brain development by mediating an anti-apoptotic effect via the activation of the MAPK/Ras signaling cascade [26]. It was also shown that pro-BDNF can trigger neuronal apoptosis by activating the c-Jun N terminal kinase (JNK) pathway via binding to the p75NTR receptor [27]. BDNF is thought to play a role in synaptic pruning and synaptic elimination and therefore contributes towards cell elimination [28]. In addition, studies have shown that neurons with low m-BDNF and high pro-BDNF levels are eliminated, supporting a role of this protein in synaptic pruning [29].

Fig. 1 Diagnostic and differential powers of these five serum proteins and ratios. ROC curves of each protein and their combination between UAS with SZ and HC
A balance is maintained between the levels of m-BDNF and pro-BDNF, which can differ in different developmental stages of the brain [30]. An elevation of pro-BDNF was observed in the early postpartum period and adolescence, while m-BDNF was found to be high in adulthood [31]. Pro-BDNF, with high levels during the developmental period, contributes to brain development by eliminating excessively mature, damaged, and malfunctioning neurons [30].

Previous studies on SZ have focused on the blood levels of these proteins with varying results reported. Pro-BDNF and m-BDNF are both active in the same pathway, are known to have opposite activities, and can also affect each other when the pathway is activated [24]. Therefore, the balance between the blood levels of these proteins is thought to be more determinant for the pathogenesis of SZ compared to individual levels. Supporting this, we observed in a previous study that this balance was shifted towards pro-BDNF in SZ patients, activating apoptosis [19].

The literature provides strong evidence that excessive synaptic pruning, particularly occurring in early adulthood and adolescence, has an important role in the development of SZ [10]. Neuroimaging studies have shown that there is a much greater loss of gray matter volume in SZ patients compared to controls [11]. Excessive apoptosis may be implicated in the pathogenesis of SZ by causing more synaptic pruning than controls. In addition, neuroimaging studies performed on UAS suggest a decrease in volume in some parts of the brain compared to HC. However, this decrease was found to not be as high as in SZ patients; therefore, volume was considered to represent an endophenotype [11].

In the current study, although we did not find a significant difference in the ratio of pro-BDNF and m-BDNF between SZ patients and their UAS, the ratio was shifted towards pro-BDNF in both groups compared to HC. This supports the data obtained from neuroimaging studies. The apoptotic-neurotrophic balance may be impaired to an extent in UAS, albeit more modestly than SZ patients, which may cause volume loss in the brain. Considering that the balance was statistically similar in SZ patients and their UAS, the ratio of pro-BDNF/m-BDNF can be considered as an endophenotypic feature for SZ.

The BDNF levels were found to be lower in the UAS group compared to those in the FEP patients; the level was significantly lower in both groups compared to that in the HC group. Additionally, the pro-BDNF values were significantly lower in the UAS compared to those in HC and FEP patients, with no significant difference found between FEP and HC groups. Activation of the tPA-BDNF pathway in a manner that favors apoptosis in SZ patients is likely to be reflected in a change in the balance compared to controls.

Serum levels of the m-BDNF receptor TrkB were found to be low in SZ patients; serum levels of the pro-BDNF receptor p75NTR were found to be statistically similar between patients and controls in some studies or lower in SZ compared to controls in others. We have previously reported lower TrkB levels in FEP patients with no significant difference in the serum levels of p75NTR, supporting the findings from the current study [19]. The p75NTR level in the current study was found to be lower in the UAS compared to that in both HC and FEP patients, while the TrkB values in the FEP group were lower than those in only the HC group. These receptors mediate the functions of pro-BDNF and m-BDNF; therefore, the balance between receptors may be similarly related to the balance between their ligands. Moreover, the activation of these receptors may vary depending on the ratio of pro-BDNF to m-BDNF. Our data indicate a higher p75NTR/TrkB ratio in FEP patients and their UAS compared to that in the HC group. A shift of this balance towards an activation of the apoptotic receptor is highly consistent with a shift in the ratio towards pro-BDNF and the pathogenesis of synaptic pruning (Fig. 2b). However, although the pro-BDNF/mature BDNF ratio was higher in UAS compared to that in HC, the absence of a significant difference between the FEP group and the HC may suggest the presence of apoptosis and thus synaptic pruning in the UAS, albeit not severe enough to cause disease (Fig. 2b).

Contrary to our expectations, m-BDNF values were the lowest in the UAS group. However, pro-BDNF values were also the lowest in the UAS group. According to these results, the need to maintain the appropriate balance between the precursor and mature forms may have resulted in the low levels of both pro-BDNF and m-BDNF. We think that this may be a balance protection mechanism. Therefore, we suggest that considering the ratio and the balance between the proteins rather than individual protein levels will provide a better interpretation of the results. These data also support the use of imaging as an endophenotype for SZ, along with evaluation of the balance between neurotrophic-apoptotic proteins.

One of the leading hypotheses in the etiology of SZ is the stress-diathesis hypothesis [32]. The stress hormones levels have been extensively investigated in patients with FEP [33]. Previous studies have reported high cortisol levels in drug-naive FEP patients [34]. Walsh et al. showed that both ACTH and cortisol levels were high in patients with FEP [35]. In the current study, we found that stress hormones were higher in the FEP group compared to those in the HC, consistent with previous findings. Additionally, there was no significant difference in cortisol levels in the UAS compared to the HC group, while ACTH levels were significantly higher in patients with SZ. Additionally, the ACTH levels of the UAS group were significantly lower than those of the FEP group. Stress hormones are directly involved in the synthesis of BDNF by inhibiting the conversion of pro-BDNF to m-BDNF [23]. In the stress-diathesis hypothesis, it has been suggested that exposure to a major stressful event can trigger SZ [33]. Thus, exposure
of SZ patients to greater stress compared to the UAS may trigger the disease. The lack of a significant increase in pro-BDNF and p75NTR levels of the UAS group in the current study may have resulted from the low cortisol levels identified. This may prevent skewing of the pro-BDNF/m-BDNF balance in the direction of apoptosis. In addition to its role in the coagulation pathway, tPA also has important functions in the brain. tPA synthesized in the oligodendrocytes and microglia specifically contributes to synaptic plasticity and neuronal regeneration [36]. Moreover, by inducing BDNF synthesis, tPA also plays a role in the formation of reward- and fear-related memory reconsolidation in the amygdala, hypothalamus, and prefrontal cortex (Fig. 2a) [37]. Previous studies have shown a decrease in tPA levels in SZ patients, which may contribute towards pathologies related to memory in SZ [36]. In the current study, no significant difference in tPA levels was identified between FEP patients and UAS or HC; the level was significantly lower in the UAS compared to that in the HC group. PAI-1 is secreted from endothelial cells and reduces the activity of tPA, playing a negative role in the formation of BDNF [23]. Although some studies found a decrease of PAI-1 in SZ patients, other studies did not find a significant difference compared to HC [38]. In the current study, tPA levels were found to be significantly lower in the UAS compared to those in the other two groups. Contrary to previous studies, tPA levels were not significantly different between FEP patients and the HC. However, the PAI-1 levels were significantly lower in both FEP and UAS groups than those in HC; moreover, it was lower in UAS compared to those in FEP patients. Considering the tPA-PAI levels together, the low PAI-1 levels in UAS may inhibit the formation of m-BDNF.

The current study has some limitations that need to be considered while evaluating the results. We did not investigate any other apoptotic or anti-apoptotic proteins and did not evaluate polymorphisms in the BDNF-related genes. Moreover, the lack of confirmation of the data in postmortem tissues and the small sample size can be counted as other limitations.

**Conclusions**

The primary finding of the current study is that the ratios of pro-BDNF/mature BDNF and p75NTR/TrkB were significantly higher in FEP patients and their UAS compared to those in the HC. The delicate balance between stimulatory and inhibitory proteins in the tPA-BDNF pathway is very important in maintaining homeostasis in the brain. A disruption of this balance towards enhanced apoptosis may trigger synaptic pruning and contribute towards the pathogenesis of SZ. It is also very important to mechanistically investigate the pathogenesis of psychosis in the UAS by establishing endophenotypes in SZ. Further research is essential to better define the possible risk of disease in UAS and to understand the possible mechanisms that protect the UAS from disease.

**Acknowledgements** We are grateful to the University of Health Sciences Turkey Research Projects Unit, Turkey, because of their financial support for the current research. In addition, our work has been awarded by the ECNP community at the 2020 ECNP congress.

**Author Contribution** UHY and SG wrote the manuscript and BGT and POM collected all the data. ZC analyzed the serum levels of the proteins. NK was responsible for overseeing and coordination of the research. All authors provided feedback and final approval on the manuscript.

**Funding** Financial support for the current research was provided by the University of Health Sciences Turkey Research Projects Unit (Date/Number: 07.03.2019/020).

**Data Availability** Available upon request.

**Declarations**

**Consent to Participate** All participants provided their written informed consent.

**Consent for Publication** N/A

**Conflict of Interest** The authors declare no competing interests.

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