Research Article

Ihsan Cetin* and Omer F. Demirel

Increased serum levels of spectrin degradation products in patients with schizophrenia

Şizofrenik bozukluğu olan hastalarda spektrin yıkılım ürünlerinin artmış serum düzeyleri

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Abstract

Objective: Under various patho-physiological and physiological conditions, spectrin breakdown reactions generate several spectrin breakdown products of 120 kDa (SBDP120) and 145 kDa (SBDP145). Previous studies indicating that there is the existence of a raised breakdown of α-spectrin in schizophrenic left superior temporal cortices. In this study, we aimed to investigate serum levels of SBDP120 and SBDP145, which has not been previously examined, and investigate their relationships with clinical parameters in patients with schizophrenia.

Methods: Forty-four patients with schizophrenia, followed by psychotic disorders unit, and 44 healthy controls, age and gender-matched volunteers with no psychiatric history, were included in this study. Sociodemographic form was applied to both groups. Turkish version of positive and negative syndrome scale (PANSS) were implemented to the patients. Serum SBDP120 and SBDP145 levels were determined by Enzyme-Linked Immuno Sorbent Assay.

Results: Serum SBDP120 ng/mL and SBDP145 ng/mL levels of the patients with schizophrenia were significantly higher than healthy controls. Even more important, serum SBDP120 levels were positively correlated with PANSS scores in patients with schizophrenia.

Conclusions: These findings may provide evidence for disturbance of neuroplasticity, membrane/cytoskeleton stability, dynamics, and remodelling in schizophrenia patients and support the neurogenerative theories for explaining the etiology of schizophrenia.

Keywords: Schizophrenia; Neurodegeneration; Cytoskeleton associated protein; SBDP120; SBDP145.

Özet

Amaç: Değişik pato-fizyolojik ve fizyolojik koşullarda, spektrin yıkımı reaksiyonları, çeşitli 120 kDa (SBDP120) ve 145 kDa (SBDP145) spektrin bozunum ürünlerini üretir. Önceki çalışmalar şizofrenide sol superior temporal kortekte α-spectrin düzeyinde artmış bir yıkımı olduğunu işaret etmektedir. Bu çalışmada, şizofreni hastalarında daha önce incelenmemiş olan SBDP120 ve SBDP145’in serum düzeylerini ve klinik parametrelerle ilişkisini araştırmayı amaçladık.


Bulgular: Şizofreni hastalarının serum SBDP120 ng/mL ve SBDP145 ng/mL düzeyleri, sağlıklı kontrollerden anlamlı derecede yüksekti. Daha da önemlisi, şizofreni hastalarında serum SBDP120 düzeyleri pozitif ve negatif sendrom ölçüçü (PANSS) skorlarıyla pozitiv yönde ilşkilidir.

Sonuç: Bu bulgular, şizofreni hastalarında nöroplastite, membran hücre iskeleti stabilitesi, dinamikleri ve yeniden şekillenmesindeki bir bozuklga ilişkin kanı
Introduction

A range of abnormalities have been defined in limbic structures of postmortem brains of patients with schizophrenia partly stemming from neuronal cytoskeletal proteins [1]. Despite significant improvements in our knowledge of schizophrenia, its pathophysiology and etiology remain obscure [2]. A role for apoptosis in schizophrenia has long been hypothesized. The decreased incidence of cancer and the decreased cancer risks in schizophrenic patients might support the notion of increased apoptotic mechanisms occurring in these patients [3]. Furthermore, the common understanding is that the elevated apoptosis levels in patients with schizophrenia and the following dendritic, synaptic and neuronal losses are because of an imbalanced apoptotic mechanism [4]. Based on such findings, investigators have suggested that the apoptosis levels of patients with schizophrenia warrant additional investigation [2–4].

Calpains and caspase-3 activities inferred from proteolysis of the cytoskeletal protein alpha-spectrin are enriched with constitutive cellular constituents playing significant biological roles in membrane/cytoskeleton stability, dynamics and remodelling [5]. The spectrin family has two α (αI and αII), and five β (βI to βV) gene coding spectrin proteins in humans [5]. Of the SPTB gene codes and the erythroid β spectrin subunits in humans [6], the SPTBN genes appear to substantially code the non-erythroid β-spectrin variants [5]. The SPTAN1 gene encodes αII-spectrin particularly expressed in neurons [7].

Spectrin catabolism is involved in multiple events, for instance; ubiquitination, intracellular trafficking/sequestration, proteolysis and release/exocytosis of breakdown products [8]. A number of studies have been conducted on proteolytic process so far, largely under acute or subacute stressful conditions in vitro and in vivo. Not only αII but also βII spectrin proteins are likely to undergo enzymatic cleavages by calpain and caspase-3 [9].

It was demonstrated that caspase-3 most swiftly divides into both αII-spectrin and βII-spectrin, and that this operation goes together with skeletal dissolution. The molecular targets of two crucial protease systems on the spectrin skeleton could interchangeably induce either sophisticated skeletal plasticity or membrane skeletal dissolution. Nevertheless, under pathologic conditions, either excessive stimulation of calpain-2 or the activation of caspase degrades βII-spectrin, with following and swift fatality [9]. At the same time, it is worth considering that this hypothesis estimates that cells are activated by restricted proteolysis of αII-spectrin, such as by calpain, which may be more susceptible to subsequent β-spectrin degradation (and therefore apoptosis). The vulnerability of βII-spectrin to calpain/caspase-mediated proteolysis is assessed in experimental and neuronal cell culture model [10]. These studies have presented the evidence that the intact 260 kDa βII-spectrin is degraded into major fragments of 80, 85, 108 and 110 kDa orchestrated by the calpain and caspase proteases both in cell culture and in vivo [11].

Alpha II-spectrin (280 kDa) is a significant substrate for caspase-3 and calpain proteases. Proteolysis of the 280 kDa alpha II spectrin by calpain I produce 150 kDa and 145 kDa fragments, indicators of excitotoxic/necrotic cell death [9] while caspase 3 digestion produced a 150i kDa SBDP (which is slightly smaller than 150 kDa SBDP150) and a characteristic 120 kDa SBDP, indicators of caspase-dependent apoptotic cell death [12, 13].

Altered spectrin and SBDP150 immunoreactivities are notified in Alzheimer disease (AD) in human brains [14], with abnormal spectrin labelling taking place in neuronal processes and axon terminals, which might stand for atypical axonal and dendritic components from sprouting neurons or accumulation of SBDPs in degenerating neurons [12]. The level of SBDPs in blood and cerebrospinal fluid (CSF) is assessed as new biomarkers for traumatic brain injury and seems to be associated with the volume of injury, calculated by tomography scans and clinical results [13].

We have few investigations about SBDP and spectrin in patients with schizophrenia. In a after death investigation carried out on patients with schizophrenia, the rate of 150–240-kDa forms of anti-a-fodrin immunoreactivities was nearly 60% higher in the left superior temporal cortices of 11 schizophrenic brains when compared to values for the left side brains of nine controls, indicating that there is an existence of a raised breakdown of α-spectrin in schizophrenics’ left superior temporal cortices [1, 15].

Previous investigations reported polymorphisms of some genes that are related to increased caspase-3 activity, higher 53 expressions and apoptosis in patients with schizophrenia [16–18]. In schizophrenic patients, although some pathologic and genetic studies have been conducted [4, 16–18], there are still limited data to reveal the role of spectrin breakdown products. Altered serum SBDPs as neurodegeneration marker in schizophrenic patients may contribute to find new evidences for explanation of
ethiopathogenesis of schizophrenia. The relation of schizophrenia and serum SBDPs has not yet been investigated, so the issue has become the subject of this study.

Materials and methods

Procedures and materials

This study was performed in psychotic disorders unit of department of psychiatry of Cerrahpaşa Medical Faculty of Istanbul University, from January to June 2015. The research protocol was certified by Ethics Committee of Cerrahpaşa Medical Faculty (23-12-2014/02-285192). Forty-four schizophrenic patients monitored by psychotic disorders unit, and 44 healthy controls were included in the study. The subjects met DSM V criteria for schizophrenia according to American Psychiatric Association [19]. Exclusion criteria were applied to the participants with a history of clinical or neurological disease, significant head injury, mental retardation and active substance abuse or dependence. Also, patients having co-morbid psychiatric illness were left out. The healthy controls were formed of 44 age-and gender-matched volunteers from hospital personnel with no substance and alcohol dependence and history of psychiatric or neurological disorder. After a full presentation of the study, participants were given informed consent forms. The study was directed in conformity with the Declaration of Helsinki, Finland. Socio-demographic form and Turkish version of positive and negative syndrome scale (PANSS) were applied to the patients; on the other hand, sociodemographic form was applied to both groups [20].

Determination of spectrin breakdown products

Blood samples were drawn into a vacutainer without anticoagulant after an overnight (≥12 h) fast. Whole blood samples were let to clot for 2 h at room temperature. The specimens were centrifuged at 1000 g for 15 min to pick up clear and yellow serum specimens for the study in question. The eppendorf tubes of serum samples were stored at –70°C after both hemolysed and lipemic blood samples were taken out.

Serum levels of SBDP120 and SBDP145 were specified by Enzyme-Linked ImmunoSorbent Assay (My Bio Source: MBS707028/MBS707178, respectively). This assay makes use of quantitatives and enzyme immunoassay technique. Antibody specific for SBDP120 or SBDP145 was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any available SBDP120 or SBDP145 was bound by means of the immobilized antibody. After any unbound substances were removed, a biotin-conjugated antibody specific for SBDP120 or SBDP145 was added to the wells. After washing process, avidin conjugated Horseradish Peroxidase was added to the wells. A substrate solution was added to the wells and colour developed. The optical density of wells was calculated at 450 nm wavelength within 10 min after stop solution was added.

Statistical analysis

Sigma Stat 3.5 and SPSS software version 15.0 (IBM Corp., Armonk, NY, USA) statistics programs were used for statistical analyses. Kolmogorov-Smirnov was used in testing normal distribution of the data. The mean values for the groups were compared to each other by employing independent t-test samples. Mann-Whitney U-test was employed since non-parametric analysis of the perpetual data did not fit the normal distribution existing between groups’ comparisons. χ²-test was employed to determine the difference in the distribution of categorical variables. The Spearman correlation test was conducted to study the connection between spectrin breakdown products and clinical parameters. Perpetual variables were verbalised as mean±SD or mean±SD/SEM, while categorical variables were in numbers. Statistical significance was set at 0.05.

Results

The study included 44 patients and 44 controls. Socio demographic characteristics and serum spectrin breakdown product levels of study groups are given in Table 1. There was not a meaningful difference between groups with regard to gender, age and marital status (p = 0.501, p = 0.647 and p = 0.312, respectively). While the mean age was 38.3 ± 9.96 years in the patient group, it was 37.56 ± 11.1 years in the control group. The mean duration of education in patient and control group subjects was 9.9 ± 5.2 and 13.6 ± 3.6 years, respectively. A significant difference was present between two groups in respect to duration of education (p = 0.000). Mean onset of age for psychosis was 23.08 ± 6.09. Serum SBDP120 ng/mL (p = 0.000; Figure 1), SBDP120 (zero/above zero, p = 0.001), SBDP 145 ng/mL (p = 0.001; Figure 2) and
SBDP145 (zero/above zero, \( p = 0.014 \)) levels of the patients with schizophrenia were significantly higher than healthy controls (Table 1).

Clinical characteristics and pharmacological treatment of patient group are given in Table 2. The mean PANSS total syndrome score in patient group was 65.8 ± 31.2 points. The mean PANSS positive syndrome score in patient groups was 14.9 ± 7.82 points. The mean PANSS negative syndrome score in patient groups was 24.01 ± 8.95 points. The mean PANSS general syndrome score in patient group was 38.7 ± 15.8 points (Table 2).

As a result of correlation analysis between spectrin breakdown products and clinical characteristics, we found that serum SBDP120 levels were significantly positively correlated with PANSS total syndrome and PANSS positive syndrome (\( \rho = 0.310^* \) and \( \rho = 0.407^{**} \); respectively) in patients with schizophrenia. Similarly, SBDP120 levels were significantly positively correlated with PANSS general syndrome (\( \rho = 0.437^{**} \); Figure 3) in patients with schizophrenia. On the other hand, the number of hospitalizations was positively correlated with numbers of electroconvulsive therapy (ECT) in schizophrenic patients (Table 3).

**Discussion**

The outcomes of our investigation ascertained that serum SBDP120 and SBDP145 levels of patients with schizophrenia were prominently higher than those of healthy ones.
Moreover, serum SBDP120 levels were positively correlated with PANSS scores in patients with schizophrenia.

Both molecules were studied in psychotic patients, but to our best knowledge, this is the first serum study to investigate SBDP120 and SBDP145 in schizophrenic patients. The most exciting fact in spectrin study comprises the identification of necessary biological functions of these proteins in brain during neurodevelopment and synaptoplasticity, and their association with particular neuronal structural domains, and findings of genetic link of them to certain inherited neurological diseases [9, 21]. In case of neuronal injury, stress and death under chronic and acute degenerative incidences, increased catabolism of cytoskeleton/membrane proteins occur, leading to the rise of their breakdown products.

During the early stage post-traumatic brain injury (TBI), SBDP145 and SBDP150 levels overwhelmingly increase, while SBDP120 levels remain elevated for a longer post-injury period. This pattern proposes that both necrotic/oncotic and apoptotic cell death mechanisms are activated after TBI, but may proceed different time courses after injury [13, 22].

On the other hand, there is much information of impaired cytoarchitecture in schizophrenic patients' brains [23, 24]. Impaired cortical organisation generally might occur due to elevated apoptosis making the life span shorter for subplate and leading to premature termination of its function as an axonal and neuronal guidance system. In a post-mortem research of schizophrenia, Akbarian and his colleagues reported that decreased interstitial cell densities in the prefrontal white matter next to the cortex [25]. Benes et al. [26] employed quantitative techniques to analyze post-mortem cortical cytoarchitecture and discovered that they substantially decreased neuronal densities in cingulate, prefrontal cortex and motor cortex in brain of schizophrenia patients.

Table 2: Clinical characteristics and pharmacological treatment of patient group.

<table>
<thead>
<tr>
<th>Patients (n = 44)</th>
<th>PANSS scores</th>
<th>Medication use</th>
<th>Other characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total syndrome</td>
<td>Positive syndrome</td>
<td>Negative syndrome</td>
</tr>
<tr>
<td></td>
<td>65.8 ± 31.2</td>
<td>14.9 ± 7.82</td>
<td>24.01 ± 8.95</td>
</tr>
</tbody>
</table>

Data are expressed as number for categorical variables and mean ± SD for continuous variables.

Table 3: Correlation between spectrin breakdown products and clinical characteristics levels in patients with schizophrenia.

<table>
<thead>
<tr>
<th></th>
<th>SBDP120</th>
<th></th>
<th>SBDP145</th>
<th></th>
<th>Number of ECT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rho</td>
<td>p-Value</td>
<td>rho</td>
<td>p-Value</td>
<td>rho</td>
<td>p-Value</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total syndrome</td>
<td>0.310*</td>
<td>0.048</td>
<td>0.005</td>
<td>0.974</td>
<td>−0.158</td>
<td>0.379</td>
</tr>
<tr>
<td>Positive syndrome</td>
<td>0.407*</td>
<td>0.007</td>
<td>0.045</td>
<td>0.779</td>
<td>0.277</td>
<td>0.277</td>
</tr>
<tr>
<td>Negative syndrome</td>
<td>0.262</td>
<td>0.094</td>
<td>0.166</td>
<td>0.295</td>
<td>−0.114</td>
<td>0.520</td>
</tr>
<tr>
<td>General syndrome</td>
<td>0.437*</td>
<td>0.004</td>
<td>0.080</td>
<td>0.615</td>
<td>0.132</td>
<td>0.455</td>
</tr>
<tr>
<td>Number of hospitalizations</td>
<td>−0.018</td>
<td>0.912</td>
<td>0.060</td>
<td>0.711</td>
<td>0.547*</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*rho, Spearman correlation coefficient. Statistically significant results are shown in bold.
schizophrenic patients. The generalised tendency towards lower neuronal densities was not taken into account for age at death, neuroleptic dose in the month before death, hypoxia related to mode of death, fixation interval or post-mortem delay. It was found out that in schizophrenic patients, the number of glia per unit volume was lower in general. These results, during neurodevelopment, might be considered as an elevated rate of apoptosis in schizophrenia [24]. By employing magnetic resonance imaging, Kulynych et al. [27] examined cortical gyrification during schizophrenia. They discovered that the gyrification index decreased great deal in the patient group, as could be estimated by elevated apoptosis shortening subplate life span during the third trimester [27].

Besides, blood and CSF SBDPs were assessed as authentic biomarkers for TBI in the clinic [19]. Changed spectrin and SBDP150 immunoreactivity were stated that in AD human brain, abnormal spectrin labelling took place in neuronal processes and axon terminals, which could prevent atypical axonal and dendritic components from germinating neurons or SBDPs’ accumulating in degenerated neurons [12].

Methamphetamine (METH) abuse, like TBI, will end up the pronecrotic calpain system activation and the pro-apoptotic caspase systems [28]. It is known that clinical and neurochemical similarities exist between METH psychosis and schizophrenia; and chronic METH intoxication model of schizophrenia in animals is summarized [29]. In these studies, it has been displayed that drug abuse is followed by functional and structural changes in brains similar to those in TBI. After drug use, calpain-1 and caspase-3 were up-regulated the same as levels of their cleavage into SBDPs, SBDP145, and SBDP120, respectively [30]. Acute METH treatment data displayed important increase in cII-spectrin protein breakdown products (SBDP120, SBDP145 and SBDP150). Accordingly, it is possible to suggest that our results are consistent with previous degradomicroscopy that were carried out in various fields of psychiatric disorders, including drug abuse (METH and ecstasy) [28–30].

In this study, we did not investigate CSF SBDP levels. Therefore, further investigations will be needed to determine if SBDP crosses the blood–brain and blood–CSF barrier and reflects SBDP levels in the CSF and brain [31]. Nevertheless, while SBDP120 and SBDP145 are typically regarded as intracellular proteins, they are, however, found out to be naturally present in extracellular biological fluids, including human CSF and blood plasma [31, 32]. CSF is in direct contact with brain interstitial fluid and, thus, great likely submits a more accurate evaluation than peripheral blood levels of spectrin breakdown products. Nonethelessness, the constant production of CSF entails that it should be exit the subarachnoid space surrounding the brain; and most likely, as CSF is discharged via the subarachnoid granulations into the venous circulation, and products released from the brain into the CSF could be transferred to blood when CSF enters the venous circulation [33].

Perhaps one of the most important finding of our study was that we demonstrated that SBDP120 levels were positively correlated with PANSS total syndrome, PANSS positive syndrome and PANSS general syndrome in patients with schizophrenia.

The PANSS is one of the most widely used methods for standardized measurement of schizophrenic core symptoms [34]. It is a well-known phenomenon that negative symptoms tend to increase with the progress of the disease [35]. N erythroid α-spectrin (fodrin), the prominent constituent of neuronal cytoskeletal proteins, is split up by the calcium-activated proteases, and the proteolytic processing has a good correlation with several neuropathologic conditions, including excitotoxicity [36] and apoptosis [13, 37]. The rate of the 150–240-kDa forms of anti-α-fodrin immunoreactivities was nearly 60% bigger in the left superior temporal cortices of 11 brains of schizophrenic patients when compared to values for the left side in nine control brains, indicating that there is an increasing breakdown of α-spectrin in left superior temporal cortices of schizophrenic patients [15, 38]. There is restricted proof about spectrin and SBDPs in the etiology of schizophrenia. We revealed raised serum levels of SBDPs in patients with schizophrenia compared with healthy controls, congruent with valuable findings of Kitamura et al. [18] proposing raised breakdown of α-spectrin in left superior temporal cortices of schizophrenic patients. Findings of Kitamura et al. [15] supported left superior temporal cortical neuroimaging findings of schizophrenic patients, finding out reduced anisotropy, raised diffusivity, and decreased volume [39].

Intact cII-spectrin is a major structural component of the cortical membrane cytoskeleton which is rich in axons and presynaptic terminals [17]. Importantly, cII-spectrin is a substantial substrate for cysteine proteases involved in necrotic (calpain) and apoptotic (caspase-3) cell death [17, 18]. Considering these aspects, we hypothesized that, measurement of 120 and 145 SBDPs by ELISA maintains the information on the relative importance of caspase or calpain-dependent cell death processes in schizophrenic patients. Schizophrenia has been argued as possessing neurodegenerative pattern in etiology; therefore, the outcomes of our investigation might uncover the probable calpain/caspase-3 activation and improved spectrin proteolysis mechanism in the etiology of schizophrenia.
On the other hand, this study has several restrictions, including limited number of samples and lack of CSF levels of SBDP 120 and 145. Secondly, cognitive tests and neuroimaging could enhance the strength of this work. Lastly, we do not have the equipment to perform the measurement of serum samples by Western blotting; therefore, it may be suggested that the results of serum ELISA repeated by western blotting, which will increase the validity of the results. Nonetheless, as far as we know, this study is the first investigation to measure serum levels of SBDPs in patients with schizophrenia and there is no similar serum study on schizophrenic patients; as a result, it may be suggested that our findings will constitute a different neurobiochemical standpoint for future investigations to be conducted out on schizophrenia; and they will provide significant contributions for the literature.

**Conclusion**

The results of our study demonstrated that serum levels SBDP120 and SBDP145 in patients with schizophrenia were prominently higher than controls. Serum levels of SBDP120 were positively correlated with PANSS scores in patients with schizophrenia. It was found out that spectrin breakdown products (SBDPs) as potential biomarkers for neurodegenerative diseases. Curr Transl Geriatr Exp Gerontol Rep 2012;1:85–93.

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**Conflict of interest statement:** The authors report no declarations of interest.

**References**


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