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Evaluation of S100B, NSE, MBP and GFAP serum levels in children with attention deficit hyperactivity disorder

Dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda serum S100B, NSE, MBP ve GFAP düzeylerinin değerlendirilmesi

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Abstract

Purpose: This study aimed to investigate serum levels of S100B protein, Neuron Specific Enolase (NSE), Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) that reflect glial and neural structure. To date, these proteins have been studied in neuropsychiatric diseases and relation with neuronal damage has been shown. However, there is no study that simultaneously evaluates the serum levels of these proteins in children with ADHD and compares them with healthy controls.

Materials and methods: 44 children with ADHD and 37 healthy volunteers participated in the study. They were selected from children and adolescents aged 6-18 years who had no history of other medical or psychiatric diseases and had not used psychotropic drugs for the last six months. Levels of proteins were assessed by enzyme-linked immunosorbent assay (ELISA).

Results: Serum levels of S100B, NSE and GFAP were found to be statistically significantly higher in the ADHD group than in the control group (p=0.012, p=0.000, p=0.001, respectively). No significant difference was found in the intergroup comparison for MBP (p=0.181).

Conclusion: Increased levels of S100B, NSE, GFAP may be an indicator of neuronal or glial changes in ADHD. Future studies combining serial measurements of these biochemical proteins with genetics and neuroimaging data are needed to evaluate the possible role of glial and neuronal damage in the etiopathogenesis of ADHD.

Keywords: ADHD, glia, astrocyte, neuroinflammation.

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Öz

Amaç: Bu çalışmanın amacı glial ve nöral yapıyı yansıtan S100B proteini, Nöron Spesifik Enolaz (NSE), Miyelin Bazik Protein (MBP) ve Glial Fibriller Asidik Protein (GFAP) serum düzeylerini araştırmaktır. Bugüne kadar bu proteinler nöropsikiyatrik hastalıklarda çalışılmış ve nöronal hasarla ilişkisi gösterilmiştir. Ancak bu proteinlerin serum düzeylerini DEHB'li çocuklarda eş zamanlı olarak değerlendirerek sağlıklı kontroller ile karşılaştıran bir çalışma mevcut değildir.

Gereç ve yöntem: Çalışmaya 44 DEHB'li ve 37 sağlıklı gönüllü katımıştır. Katılımcılar, başka tıbbi veya psikiyatrik hastalık öyküsü olmayan ve son altı aydır psikotropik ilaç kullanmayan 6-18 yaş arası çocuk ve gençler arasından seçilmiştir. Serum protein seviyeleri enzim-bağlı immünosorbent yöntemi (ELISA) ile değerlendirilmiştir.

Bulgular: S100B, NSE ve GFAP serum düzeyleri, DEHB grubunda kontrol grubundan istatistiksel olarak anlamlı düzeyde yüksek bulunmuştur (sırasıyla p=0,012, p=0,000, p=0,001). MBP için gruplar arası karşılaştırmada anlamlılık düzeyinde bir farklılık saptanmamıştır (p=0,181).

Sonuç: S100B, NSE, GFAP'nin yükselmiş düzeyleri DEHB'deki nöronal veya glial değişikliklerin bir göstergesi olabilir. DEHB etiyopatogenezinde glial ve nöronal hasarın olası rolünü değerlendirmek için bu biyokimyasal proteinlerin seri ölçümlerini genetik ve nörogörüntüleme verileriyle birleştiren gelecek çalışmalara ihtiyaç vardır.

Anahtar kelimeler: DEHB, glia, astrosit, nöroinflamasyon.

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Introduction

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by inattention, hyperactivity, and impulsivity. It has a multifactorial etiology with no single cause. Mainly biological causes play a role in the etiology of ADHD, and some environmental and psychosocial adversities are related to the disorder [1, 2]. In a meta-analysis, ADHD was found to be one of the most heritable psychiatric disorders with an average heritability of 76% [3].

As Farone stated, the etiology and pathogenesis of ADHD are not clearly defined, so more valid diagnoses would be welcomed [4]. Biomarkers are supposed to be good candidates at this point. A biomarker refers to a characteristic that can be objectively measured and assessed to indicate normal biological functions, disease-related processes, or the body's response to a therapeutic treatment [5]. Although various biomarkers related to neurophysiology, neurochemistry, neuroimaging, and genetics have been identified with small to moderate effects in ADHD, a definitive biomarker for its diagnosis has not yet been established [6].

Brain-specific proteins such as S100B (S100Beta), NSE (Neuron Specific Enolase), GFAP (Glial Fibrillary Acidic Protein), and MBP (Myelin Basic Protein) are not found in other tissues and can be easily measured in the blood. These proteins may provide information about the active status of brain regions with structural and functional damage, the severity of the disease, and the prognosis of the patient [7].

The S100B protein family is responsible for protein phosphorylation, cell growth and change, regulation of transcription factors and enzymes, enzyme activities, inflammatory cell response, and Ca+2 metabolism [8]. The increase in S100B in serum in traumatic brain injury, Down Syndrome, Alzheimer's Disease, manic attacks, and schizophrenia is generally associated with neural toxicity, and this has led some researchers to call it as "CRP of the brain" [9]. S100B overexpression has been detected in children with cerebral palsy and developmental delay [10]. It has also been stated that S100B may be involved in the pathogenesis of psychiatric diseases related to its neurotrophic effect [11]. It has also been found to be elevated in autism spectrum disorder (ASD) [12, 13]. A study involving 360 ADHD-probands reported that S100B moderates the relationship between low birth weight and hyperactivity and impulsivity symptoms [14]. Another study reported that maternal smoking history was associated with increased S100B levels in children with ADHD [15]. Liu et al. [16] reported a positive association between S100B levels and ADHD symptoms in a group of ADHD children with lead exposure.

NSE (Neuron Specific Enolase) increases in blood and cerebrospinal fluid (CSF) secondary to structural damage in neurons. It is secreted from neuroendocrine cells in the central nervous system and the periphery [17]. Since it is involved in ATP synthesis, it is of vital importance for the excitability of the neuronal membrane [18]. NSE has been investigated in many chronic neurological diseases. It has been proven to be useful in the diagnosis of various conditions, including traumatic brain injury, epilepsy, intracerebral hemorrhage, ischemic stroke, Alzheimer's disease, Creutzfeldt-Jakob syndrome, delirium, and Guillain-Barré syndrome." [19]. Wiener et al. [20] investigated NSE in bipolar disorder and depression patients and reported lower levels than controls. The authors mentioned the role of NSE in energy metabolism and emphasized more permanent and irreversible damage by disease progression leading to loss of neurons and brain volume. They attributed reduced serum levels of NSE to diminished synaptic connectivity and impaired structural plasticity that is present in patients with mood disorders. ASD patients have been researched regarding NSE levels, and increased levels [21, 22] or no difference [23] have been reported in the ASD groups in comparison to controls. Regarding ADHD, the only case-control study researching serum NSE levels included internet-addicted child and adolescent patients, and the ADHD group had higher levels of NSE with S-100B [24].

GFAP and MBP, which show neuronal, astrocytic, and oligodendroglia damage in neuropsychiatric diseases, are other potential neuromarkers [8]. MBP is in the myelin sheath and is synthesized only by oligodendrocytes and Schwann cells during active demyelination. In the presence of neuroglial damage, MBP

levels increase in serum [25]. Its increment levels in some neurological diseases, such as Multiple Sclerosis, provide valuable information about the severity of the disease [25]. GFAP is an acidic cytoskeleton protein and a basic intermediate filament in neurons [26]. It has high sensitivity for the status of the neuroglial structure and is important in the central nervous system neurodegeneration and damage [26]. GFAP was again found to be high and clinically significant in children diagnosed with infantile autism, and it has been stated that elevated levels of GFAP suggest gliosis and nonspecific brain damage in children with autism [27]. A case-control study in ADHD-diagnosed patients also found higher levels of GFAP compared to controls [28].

Although S100B has been studied in ADHD, albeit in small numbers, there are very scarce case-control studies on NSE, GFAP, and MBP. This study aimed to measure these markers in ADHD-diagnosed children and adolescents and to compare the marker levels with healthy controls. We hypothesized that the levels of S100B, NSE, GFAP, and MBP would have increased in the ADHD group in comparison to healthy subjects. Secondly, we aimed to search the relation of these markers with each other in ADHD patients and with neurocognitive test results. Our second hypothesis was that biomarker levels would be positively correlated with each other and would show a significant relationship with neurocognitive test results.

Materials and methods

The research was conducted at the Department of Child and Adolescent Psychiatry, Pamukkale University Faculty of Medicine, between November 2018 and June 2019. The current study is a cross-sectional case-control study comparing serum S100B, NSE, MBP, and GFAP levels of ADHD-diagnosed children and adolescents with healthy controls.

Within the scope of the study, children and adolescents between the ages of 6 and 18 who applied to Pamukkale University Faculty of Medicine Child and Adolescent Psychiatry Outpatient Polyclinics with ADHD symptoms were evaluated. A total of 44 voluntary children and adolescents who were diagnosed with ADHD according to Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) diagnostic criteria and met the inclusion criteria for the study were recruited for the study to form the ADHD group. From the same outpatient polyclinics, 37 voluntarily similar ages of children who were not diagnosed with any psychiatric disorder composed the control group.

Having received psychotropic medication within the last 6 months, having an infection history and any medication use within the last week, having a chronic medical disease/ continuous medication use, having symptoms that would suggest mental retardation clinically, being unwilling to participate in the study, not being able to complete the necessary evaluations and tests, or the participant or their parent giving up participating in the study were accepted as exclusion criteria for both groups. For the ADHD group, having a diagnosis of a psychiatric disorder other than ADHD, and for the control group, having been diagnosed with any psychiatric disorder according to DSM-V diagnostic criteria at the end of the clinical assessment were accepted as exclusion criteria.

Participants who agreed to participate in the study and their parents were clinically interviewed, study scales were filled in, computer-based psychometric assessment tests were applied, and 10 cc of venous blood was taken for laboratory analysis.

In the clinical evaluation phase, psychiatric interviews based on DSM-V were conducted to evaluate ADHD and other possible psychiatric diagnoses with all children and adolescents and their families in the ADHD and control groups. During the interview, the sociodemographic data form and the Turgay DSM-IV Based Child and Adolescent Disruptive Behaviors Disorders Screening and Rating Scale were filled out by the parents. In addition, two computer-based neurocognitive tests, the Psychology Experiment Building Language (PEBL)-Berg's Wisconsin Card Sorting Test, which is the short computer version of the Wisconsin Card Sorting Test, and the Stroop Victoria test, were applied to the children and adolescents during the interview. All interviews and test applications were completed by the same researcher.

All children, adolescents, and their families participating in the study were informed about the study in accordance with the Declaration of Helsinki. Written consent was obtained from both parents and children. Before the study, approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee in the meeting dated 29.05.2018 and numbered 11, with the number 601167787-020/40438 (additional ethical approval for a correction application was received in the meeting dated 21.01.2020 and numbered 02, with the number 601167787-020/10336). This study was supported by Pamukkale University Scientific Research Projects Commission with the decision numbered 2018TIPF038.

Study instruments

Turgay DSM-IV-based child and adolescent disruptive behavior disorders screening and rating scale

The Turgay DSM-IV-Based Child and Adolescent Disruptive Behavior Disorders Screening and Rating Scale was developed by Turgay (1994) [29], based on DSM-IV diagnostic criteria. It consists of 9 items for inattentiveness, 6 items for hyperactivity, 3 items for impulsivity, 8 items for oppositional defiant disorder, and 15 items for conduct disorder. Each item has four options ranging from none to a lot. The validity and reliability study of the scale was conducted by Ercan et al. [30].

Wisconsin Card Sorting Test (WCST)

It was created by Grant and Berg in 1948 [31] and developed by Heaton in 1981 [32]. Turkish standardization was made by Karakaş [33]. The WCST is a frontal lobe test associated with attention, feature identification, working memory, executive functions, conceptualization, abstract thinking, and especially deperseveration. It assesses the individual's response to changing conditions and the problem-solving strategies.

WCST consists of two decks of cards, each consisting of 64 response cards and 4 stimulus cards. Each card contains shapes (plus, circle, star, and triangle) in different colors (red, yellow, blue, and green) and quantities (one, two, three, and four). The task that the individual must do in WCST is to match the response cards with the stimulus cards that he/she deems appropriate. In the test, the number of completed categories, the total number of correct answers, the total number of errors, the total number of perseverative responses, the total number of perseverative errors, the number of nonperseverative errors, the unique error, the number of trials used to complete the first category, the learning to learn score, the number of conceptual level responses, and the percentage of conceptual level responses are calculated by the computer program.

Stroop color and word test, Victorian version

Originally developed by Stroop (1935) [34], various forms of the test have subsequently been developed. The Turkish standardization was made by Karakaş [33] and Kilic et al. [35]. There are many different versions of the Stroop test, including the Victorian version used in this study. The Stroop test measures the ability to change perceptual setups in response to changing demands and under a "disruptive stimulus", the ability to suppress a habitual behavior pattern and engage in an unusual behavior, and additionally focused attention. The Stroop test provides a timed measure of selective attention and cognitive flexibility.

The test is composed of Part D (dots, shapes), W (words, neutral/non-colored words) and C (colors). The time to complete the tasks and the number of errors made are recorded by the PEBL program. The first and second parts of the test are used to measure cognitive speed, while the third part of the test is used to measure response inhibition. The computer program (PEBL)-based administration of the test requires the participant to correct the error if an error is made before moving on to the next item. This is reflected in the completion time of the test. The test was administered to children and adolescents in the consultation room of the outpatient clinic via the computer program (PEBL).

Biochemical evaluation

Ten cc of venous blood was taken from the antecubital vein of participants after 12 hours of fasting. The samples were kept at room temperature for approximately 15 minutes in the Physiology Laboratories of Pamukkale

University Faculty of Medicine and then centrifuged at 7240 rpm for 6 minutes. The samples obtained were stored at -80 °C until the evaluation time for S100B, NSE, GFAP, and MBP levels. The study was conducted with YLBiont ELISA kits (Shanghai YL Biotech Co., Ltd.).

In the laboratory analysis phase, first, all collected samples and kits were brought to room temperature. After the standard solutions of the kits, Chromagen A-B, and antigen- and antibody-containing chemicals were prepared, standards and samples were placed in the wells in the plate. Then, the samples were colored by following the steps explained in the manual. After the color formation was observed, the absorbance values of the wells at 450 nanometers (nm) were read, and the results were recorded. Concentrations were calculated using the serum absorbance values. The values were in nanogram/milliliter (ng/ml) units, except for MBP. MBP was in picograms/ml.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences version 18 (SPSS Inc., Chicago, IL, USA). Pearson Chi-square test (X^2) was used to compare categorical data. The Shapiro-Wilk test was used to evaluate whether the distribution of continuous variables was normal. Homogeneity of variances was determined by Levene test. In the comparison of two groups of numerical values, Student t-test was used if continuous variables showed normal distribution, and the Mann-Whitney U test was used if they did not show normal distribution. In examining the relationship between continuous variables, the Pearson correlation test was used for those with normal distribution and the Spearman correlation test for those without normal distribution. Statistical significance value was accepted as $p<0.05$.

Results

In the ADHD group, 10 (22.73%) participants were female and 34 (77.27%) were male; in the control group, 15 (40.54%) participants were female and 22 (59.46%) were male. There was no statistically significant difference between the ADHD group and the control group in terms of gender ($p=0.084$). The mean age of the ADHD group was 9.95±3.09 years, and the mean age of the control group was 10.61±0.92 years. No statistically significant difference was found between the two groups in terms of age ($p=0.292$).

The ADHD and control groups were compared in terms of the biochemical parameters analyzed in the study. Accordingly, the mean S100B values of the children in the ADHD group were found to be 33.89±16.07 ng/L, and the mean S100B values of the children in the control group were found to be 26.05±4.43 ng/L. When the ADHD and control groups were compared in terms of S100B values, it was seen that S100B values of the ADHD-diagnosed participants were higher than the values of the control group, and a statistically significant difference was recorded ($p=0.012$).

The mean NSE values of the ADHD group were found to be 3.73±1.37 ng/ml, and the mean NSE values of the control group were found to be 2.73±0.71 ng/ml. When the ADHD and control groups were compared in terms of NSE values, it was seen that the NSE values of the ADHD group were higher than the control group, and a statistically significant difference was found between them ($p<0.001$).

The mean GFAP values of the ADHD group were determined as 1.33±0.56 ng/ml, and the mean of the control group was determined as 1±0.25 ng/ml. When the ADHD and control groups were compared in terms of GFAP values, it was seen that the values of the ADHD group were higher, and a statistically significant difference was present ($p=0.01$).

The mean serum MBP values of the ADHD group were determined as 644.91±395.87 ng/L, and the mean MBP values of the control group were determined as 539.48±246.29 ng/L. When the ADHD and control groups were compared in terms of MBP values, although the values of the ADHD-diagnosed participants were higher than the control group, no statistically significant difference was found ($p=0.181$) (Table 1).

Table 1. Comparison of ADHD and Control Groups in terms of S100B, NSE, GFAP and MBP values

	ADHD Group		Control Group		*z*	*p*
	Mean±SD	**Med (min-max)**	**Mean±SD**	**Med (min-max)**		
S100B	33.89±16.07	28 (21.47-7.17)	26.05±4.43	25.55 (16.96-37.26)	-2.503	0.012*
NSE	3.73±1.37	3.44 (1.89-8.23)	2.73±0.71	2.74 (0-4.25)	-3.958	0.000*
GFAP	1.33±0.56	1.11 (0.51- 3.15)	1±0.25	0.94 (0.57-1.75)	-3.337	0.001*
MBP	644.91±395.87	519.75 (277.1-2143)	539.48±246.29	494.6 (303.9-1349)	-1.337	0.181

z: Mann Whitney U test, *p<0.05 is statistically significant, Med: median, min: minimum, max: maximum, SD: standard deviation
S100B: S100 Beta, NSE: neuron specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

When the relation between biochemical parameters in the ADHD group was examined, positive correlations ranging from small to large levels (statistically significant r values ranged between 0.3 and 0.7; r values between 0.2 and 0.3 were accepted as low, between 04. and 0.5 as medium, and greater than 0.6 as large correlations) were detected. Table 2 presents the relationship between S100B, NSE, GFAP, and MBP values.

Table 2. Correlations between S100B, NSE, GFAP and MBP values measured in the ADHD group

		S100B	**NSE**	**GFAP**	**MBP**
S100B	**r**	1.00	0.765[a]	0.551[a]	0.324[b]
	p		0.000*	0.000*	0.032*
NSE	**r**		1.000	0.487[a]	0.392[a]
	p			0.001*	0.009*
GFAP	**r**			1.000	0.080[a]
	p				0.605
MBP	**r**				1.000
	p				

[a]: Spearman correlation test, [b]: Pearson correlation test, *p<0.05 is statistically significant, S100B: S100Beta, NSE: neuron specific enolase
GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

Within the scope of the study, the Wisconsin Card Sorting Test (WCST) and Stroop Victoria tests were applied to the ADHD and control groups of participants, and the possible correlations between the test and subtest results and biochemical analysis values were investigated. Accordingly, the neurocognitive test analysis results of the ADHD group were generally unfavorable in comparison to the control group, and some of the findings were at statistical significance level (Table 3). No clinically important medium or large correlation was found between the neurocognitive test results and S100B, NSE, GFAP, and MBP values. Table 4 presents the statistically significant correlations in groups.

Table 3. Comparison of ADHD and Control Groups in terms of Stroop Victoria and Wisconsin Card Sorting Test (WCST) results

	ADHD Group		Control Group			
WCST	**Mean±SD**	**Med (min-max)**	**Mean±SD**	**Med (min-max)**	***z***	***p***
Number of completed categories	2.02±1.5	2 (0-5)	3.06±1.48	4 (0-5)	-2.906	0.004*
Total number of correct answers	43.98±19.4	42 (17-100)	49.61±15.64	51 (23-100)	-2.265	0.023*
Total number of errors	28.47±13.35	27 (8-64)	18.27±9.7	14 (7-41)	-3.461	0.001*
Total number of perseverative responses	22.12±14.7	21 (0-58)	23±11.43	22 (0-52)	-0.252	0.801
Total number of perseverative errors	12.81±10.39	10 (0-38)	11.61±8.19	9 (0-39)	-0.283	0.777
Number of non-perseverative errors	15.28±12.04	12 (2-47)	6.67±6.53	4 (0-28)	-3.911	0.000*
Unique error	5.35±7.23	2 (0-26)	1.52±1.8	1 (0-8)	-2.424	0.015*
Number of trials used to complete the first category	16.09±11.94	13 (0-59)	15.12±10.88	11 (0-55)	-1.041	0.298
Learning to learn score	4.27±5.85	4.12 (-7.95-14.5)	4.35±5.7	3.1 (-1.96- 23.8)	-1.440	0.150
Number of conceptual level responses	35.53±20.05	36 (7-87)	43.58±16.43	44 (13-91)	-2.207	0.027*
Percentage of conceptual level responses	47.87±22.05	50 (10.9-84.4)	63.81±17.45	68.8 (20.3-84.4)	-3.188	0.001*
Stroop test						
Part D time	84.23±43.71	74.44 (26.03-210.77)	68.77±29.29	62.67 (30.17-146.81)	-1.359	0.034*
Part D errors	27.79±6.36	26 (24-60)	25.73±1.63	25 (24-31)	-0.732	0.336
Part W time	78.9±51.34	71.91 (22.78-296.61)	54.14±25.51	48.26 (30.1-148.7)	-2.734	0.001*
Part W errors	26.79±3.97	25 (24-41)	25.97±2.3	25 (24-34)	-0.032	0.859
Part C time	97.19±63.68	77.5 (24.68-311.65)	71.75±41.48	62.28 (36.15-228.63)	-1.867	0.012*
Part C errors	29.58±6.69	27 (24-59)	27.61±3.98	26 (24-42)	-0.936	0.256

z=Mann Whitney U test, **p*<0.05 is statistically significant, WCST: Wisconsin Card Sorting Test, Med: median, min: minimum, max: maximum SD: standard deviation

Table 4. Significant correlations between biochemical parameters and neurocognitive test results in the study groups

	r	*p*
Whole group		
NSE- WCST non-perseverative errors	0.309	0.007*
GFAP- WCST number of completed categories	-0.271	0.018*
GFAP- WCST number of correct answers	-0.239	0.037*
GFAP- WCST number of conceptual level responses	-0.249	0.030*
MBP- WCST learning to learn score	0.353	0.032*
MBP-Stroop Part C errors	-0.242	0.036*
ADHD group		
S100B- WCST learning to learn score	0.327	0.032*
Control group		
MBP- WCST perseverative errors	-0.369	0.035*

r: Spearman correlation test, **p*<0.05 is statistically significant, WCST: Wisconsin Card Sorting Test, S100B: S100 Beta
NSE: neuron specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

Discussion

Neuronal tissue-specific proteins such as S100B, NSE, GFAP, and MBP can be easily measured in the blood and provide information about the active status of brain regions with structural and functional damage, the severity of the disease, and the prognosis of the patient [7]. The levels of these proteins have been investigated in many chronic neurological diseases and some psychiatric diseases. In the literature, there are very limited studies on these markers conducted with ADHD patients, and these markers have not been studied collectively in ADHD. In our study, it was hypothesized that S100B, NSE, GFAP, MBP levels would be increased in children and adolescents with ADHD compared to the control group, and this hypothesis was confirmed in other proteins except MBP. In addition, we analyzed the interrelation among these biomarkers in ADHD patients and recorded a medium or large relationship in most assessments. It is known that neurocognitive test performances of individuals with ADHD are generally lower than those of the healthy controls. Based on this, it was thought that increased marker levels may be associated with unfavorable test results, but no meaningful evidence was obtained in this direction. Future studies with larger samples may shed light on this issue. The results of biochemical parameters are discussed below in light of literature information.

The S100B protein family is mainly found in astrocytes and secreted by these cells [8]. Elevations of S100B in peripheral body fluids in various neurological and psychiatric conditions are attributed to brain damage or dysfunction and increased blood-brain barrier permeability [36]. A recent study reviewed research that investigated peripheral S100B levels in psychiatric disorders: schizophrenia, depressive disorder, bipolar disorder, and ADHD. The authors concluded that mostly elevated S100B levels across disorders had been reported, but the results are inconsistent, and alterations in S100B peripheral levels do not seem to be disease specific [37].

There are very limited studies in the literature comparing S100B levels between ADHD and control cases. A study by Oades et al. [38] compared 21 ADHD medication-naive cases with 21 healthy controls and reported no marked group differences in levels of S100B. On the contrary, a recent study by Ouadih Moran et al. [39] reported higher S100 B levels in medication-naive ADHD children and adolescents than in the healthy controls, which is parallel to our result. The authors proposed that this finding of elevated S100B levels in the

ADHD group strengthened the hypothesis of glial damage within the mechanisms preceding the catecholaminergic disruption responsible for ADHD [39]. Another recent study also reported that elevated S100B levels in children and adolescents with ADHD and internet addiction compared to healthy controls, and S100B was correlated with low sleep quality [24]. Our findings support these two studies' reports of elevated S100B in the ADHD groups. As a neurodevelopmental disorder, in ADHD, increased levels of a well-known peripheral brain damage marker may be an indication of neurotoxic processes that take place in etiopathogenesis.

Another neuronal cell-specific biomarker, NSE, is localized in the cytoplasm of the neuron and increases in CSF and serum in case of neuronal damage [40]. NSE has not been studied effectively in the ADHD field. A study that included children with traumatic brain injury reported an association between higher scores on inattention, hyperactivity/impulsivity, and executive functioning scales [41]. The only case-control study included internet-addicted ADHD patients and found elevated NSE levels in comparison to healthy controls [24]. ASD, being another neurodevelopmental disorder, has had relatively more NSE research compared to ADHD, although it is still scarce. A retrospective study examining newborn blood samples from children with autism reported increased NSE levels compared to healthy controls [42]. Two more recent studies showed increased levels of NSE in ASD children compared to controls [21, 22], in contrast to the study by Esnafaoglu et al. [23], which reported no difference. Although there are very limited numbers of studies investigating NSE levels in neurodevelopmental disorders, our findings support the increased levels of NSE in ADHD-diagnosed children and adolescents.

GFAP is a major part of astrocyte intermediate filaments [6]. Astrocytes, a type of glial cell, are cells that play many roles in brain development, healthy survival of neurons, and brain homeostasis. GFAP mainly provides resistance of brain tissue to mechanical stress and plays a role in astrocyte functions such as cell migration and motility. In the presence of damage in the central nervous system, there are changes in the morphology and function of astrocytes, and astrocytes increase GFAP expression in response to neuronal damage [43]. GFAP, such as S100B, is an established indicator of astrogliosis in neuropathology [36]. A recent review study assessed GFAP in neurological disorders, including ASD, and suggested elevated GFAP levels as a valuable body fluid biomarker in the evaluation of different neurological diseases [44]. In regard to ADHD, animal [45, 46] and human studies have been conducted [28, 47]. A case-control study by Cetin et al. [28] found higher GFAP levels in the ADHD group than the control group, which is parallel to our study result [35]. Collectively, the study results point out astroglial involvement in rat models of ADHD or increased levels of GFAP in ADHD patients. Our study finding of elevated GFAP in the ADHD group of children supports the astroglial dysfunction in ADHD.

MBP is in the myelin sheath. It is found in both the central and peripheral nervous systems. It increases in serum only during active demyelination and in the presence of neuroglial damage [6]. Elevation in MBP levels in serum is not expected while myelination continues; however, MBP becomes detectable in the serum in cases of demyelinating processes and disruption of the blood-brain barrier [25]. DTI (diffusion tensor imaging) studies have shown that in the white matter of ADHD patients, there is delayed maturation and microstructural anomalies [48]. Shaw et al. [49] reported in their DTI study conducted with adult ADHD patients that white matter dysfunction was present only in the group with ongoing symptoms; white matter was alike with the control group in ADHD patients whose symptoms remitted in adulthood. Considering that ADHD is a neurodevelopmental disorder that presents symptoms starting from childhood, and it does not exhibit clinical features that are progressive and progress with biological destruction (such as in the demyelinating process in multiple sclerosis); it seems understandable that there was no statistically significant increase in MBP levels -an indicator of a demyelinating process-, between the ADHD and control groups in our study. No study evaluating MBP levels in ADHD is found in the literature. A very recent study reported that the level of myelin autoantibodies in the context of autoimmunity did not predict the diagnosis of ADHD [50].

In our study, possible interrelations of neuron-specific proteins were also assessed among ADHD children and adolescents. Accordingly, it was found that the protein couples, except GFAP-MBP, were moderately or highly correlated with each other. This finding can be considered a consistency with the other results of the study and a support for the neurobiological basis of ADHD.

As a last point, we compared the neurocognitive performance of ADHD patients with healthy controls with WCST and Stroop tests. As expected, ADHD children showed worse performance than the controls in general. However, we could not find a meaningful association between neurocognitive scores and biochemical markers, although some correlations were recorded. Future studies with greater sample sizes may provide evidence for possible relations.

When evaluating the results of our study, it is necessary to consider some limitations. The case and control groups consisted of relatively small numbers of children and adolescents. Although systemic diseases were excluded with detailed anamnesis, acute phase reactant or disease marker measurements such as CRP and sedimentation, were not assessed. In addition, although clinically normal IQ participants were included in both groups, intelligence was not measured with a psychometric test, and the groups were not matched in terms of intelligence score. It would be useful to consider the ADHD subtypes separately. The fact that the ADHD group was composed of medication-naïve children and adolescents and that additional psychiatric and medical diseases were excluded in both groups was a strength of the study.

Conclusively, we suggest that the increase in S100B, NSE, and GFAP levels may reflect microglial or astroglial changes as well as the presence of neuroinflammation in ADHD. To more accurately evaluate the possible role of glial and neuronal tissue in the etiopathogenesis of ADHD, further studies with serial measurements that combine genetic research and neuroimaging findings are required. The data to be obtained may contribute to a holistic understanding of the etiopathogenesis of ADHD.

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Authors contributions: H.C.Y. and B.K.B. have constructed the main idea and hypothesis of the study. They developed the theory and arranged/ edited the material and method section. H.C.Y. collected the study data and has done the evaluation of the data with E.B.U. in the Results section. E.B.U. conducted the laborotory analysis and contributed to the Materials and Methods section. The discussion section of the article is was written by H.C.Y. and B.K.B.

B.K.B. and H.C.Y. reviewed, corrected and approved the study. In addition, all authors discussed the entire study and approved the final version.

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