

High Serum Levels of Serum 100 Beta Protein, Neuron-specific Enolase, Tau, Active Caspase-3, M30 and M65 in Children with Autism Spectrum Disorders

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Objective: The purpose of this study was therefore to investigate whether neuronal, axonal, and glial cell markers (Neuron-specific enolase [NSE], tau, serum 100 beta protein [S100B], respectively) and apoptosis markers (active caspase 3, M30, M65) and whether these parameters can be used as diagnostic biomarkers in autism spectrum disorders (ASD).

Methods: This study measured the serum S100B, NSE, tau, active caspase 3, M30, and M65 levels in 43 patients with ASD (aged 3–12 years) and in 41 age- and sex-matched healthy controls. ASD severity was rated using the Childhood Autism Rating Scale. The serum levels were determined in the biochemistry laboratory using the ELISA technique. The receiver operator characteristics curve method was employed to evaluate the accuracy of the parameters in diagnosing ASD.

Results: Serum S100B, tau, NSE, active caspase-3, M30, and M65 levels were significantly higher in the patient group than in the control group ($p < 0.001$, $p = 0.002$, $p = 0.002$, $p = 0.005$, $p < 0.001$, and $p = 0.004$, respectively). The cut-off value of S100B was 48.085 pg/ml (sensitivity: 74.4%, specificity: 80.5%, areas under the curve: 0.879, $p < 0.001$).

Conclusion: Apoptosis increased in children with ASD, and neuronal, axonal, and glial cell injury was observed. In addition, S100B may be an important diagnostic biomarker in patients with ASD. Apoptosis, and neuronal, axonal and astrocyte pathologies may play a significant role in the pathogenesis of ASD, and further studies are now required to confirm this.

KEY WORDS: Autism spectrum disorder; Serum 100 beta protein; Neuron-specific enolase; Tau protein; Apoptosis; Caspase-3.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition. A significant rise in the prevalence of the condition in recent years has increased the importance of clarifying its pathogenesis. Genetic factors occupy an important place in the etiology, and oxidative stress, inflammation, mitochondrial dysfunction, immune

dysregulation and environmental factors have also been linked to ASD [1]. Diagnosis of ASD is based on clinical evaluation, and no biochemical diagnostic methods are currently available. It is therefore difficult to determine the risk of the disorder developing and/or to identify it before the onset of symptoms. Due to the importance of early diagnosis and educational therapies in terms of positive prognosis, identification before the onset of symptoms is crucial. Although antioxidant enzyme levels, oxidative stress markers, and cytokines have been investigated as peripheral biomarkers of ASD, no specific biomarker has to date been identified [2,3].

Tau protein plays a particularly important role in the stabilization of axonal microtubules in central neurons and in the maintenance of axonal transport [4]. Aggregation

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occurs in case of hyperphosphorylation, and intra-neuronal accumulation may emerge, thus leading to axonal transport impairment, synaptic miscommunication, and neurodegeneration [5,6]. Additionally, in the presence of axonal injury tau passes into the extracellular space, and from there into cerebrospinal fluid [7]. *Amyloid beta* peptide, which plays an important role in Alzheimer's disease, has been shown to require Tau protein to impair hippocampal synaptic plasticity [8]. In addition, candidate gene studies concerning ASD have reported that proteins involved in synaptic mechanisms are linked to ASD risk genes [9]. One study showed increased expression of cathepsin D (a lysosomal protease), responsible for the formation of tau fragments predisposed to abnormal phosphorylation, in patients with ASD [10]. Moreover, alteration has been determined in the phosphorylated:unphosphorylated tau ratio, suggestive of abnormal microtubule depolymerization, in the hippocampus of autistic-like mouse strain C58/J [11]. Lower serum tau protein levels have also been determined in an autistic group compared to a control group [12]. Evaluation of the relation between Tau protein and ASD is particularly important considering that synaptic and myelin dysfunction is present in the pathogenesis of ASD, and that microtubules and microtubule-associated proteins are important regulators of myelination, synaptic formation, and neuron production [13].

Serum 100 beta protein (S100B), which also plays a role in Tau protein phosphorylation [14], may be present at low levels in the blood of healthy individuals, but serum levels have been shown to increase in primary and secondary cerebral injury [15,16]. The effect of S100B is dose-dependent. At low, nanomolar, concentrations, it exhibits a neuroprotective effect [17] and plays a role in the regeneration of injured nerves [18]. At higher, micromolar, concentrations, it increases the production of reactive oxygen species (ROS) in neurons, and induces nitric oxide-dependent death of astrocytes and neurons [19]. High serum S100B levels have also been linked to various psychiatric diseases, such as schizophrenia [20] and mood disorders [21]. Studies investigating the relation between ASD and S100B in the last decade have observed high serum [22,23] and plasma [24] S100B levels.

Neuron-specific enolase (NSE) is localized in neuron cytoplasm and peripheral neuroendocrine cells [25]. An increase in NSE levels in CSF has been shown in case of

neuronal injury such as stroke, head trauma, multiple sclerosis, Alzheimer's disease, and epileptic seizure [26]. Since NSE is a cytosolic enzyme, it may only appear when the cell is injured [27], and its levels have been shown to be associated with the degree of cerebral injury [28]. Wang *et al.* [29] reported hypermethylation of the NSE gene in 14.5% of autistic children, as well as decreased NSE RNA expression. Higher neonatal serum NSE has also been reported in patients with ASD compared to controls [30].

Caspases are cysteine proteases found in cytoplasm and are involved in apoptosis (programmed cell death) [31]. Cytokeratin 18 (CK-18), a cytoskeleton protein released by apoptotic cells, is broken down into M30, an apoptosis marker, with caspase activation. M65 comprises both cleaved (apoptosis) and uncleaved (necrosis) CK-18 and is used as a marker for total cell death [32]. Apoptosis is thought to play a role in the pathogenesis of neurodegenerative diseases such as Alzheimer's, Hutchinson's, Parkinson's, and amyotrophic lateral sclerosis, and also of AIDS and autoimmune diseases [33]. Apoptotic signals (such as increased cellular oxidative stress or hypoxia) deriving from inside the cell result in apoptosome. Apoptosome triggers apoptosis by activating caspase-3 [34]. Apoptosis can be determined through identification of various activations known to be apoptosis-specific (such as active caspase-3 assay) at the molecular level. The last two decades have seen an increase in studies investigating the role of apoptosis in the pathogenesis of ASD. Decreased antiapoptotic Bcl-2 protein [35] and increased cathepsin D, caspase-3 [10] and p53 [35] levels have been shown in the cerebella of autistic subjects. Decreased Bcl-2 expression and increased cathepsin D mRNA and protein have been reported in autistic lymphoblasts, and increased apoptosis has been implicated in the pathogenesis of ASD [10]. An increase in this pathological activation in apoptosis in patients with ASD has also been potentially associated with neuro-anatomic abnormalities [36].

S100B, tau and NSE levels have previously been investigated separately in patients with ASD, but not in the same patient group. Additionally, although different parameters associated with apoptosis have been investigated, there has been no previous evaluation of the markers M30 and M65 in ASD patients. Considering the presence of synapse and myelin dysfunction in the patho-

genesis of ASD, our aim was to assess levels of tau, which is involved in myelination and synaptic formation. We also planned to examine the relationship with S100B-tau in patients diagnosed with ASD by evaluating levels of S100B that causes tau phosphorylation in the same patient group. In addition, since S100B increases ROS at high concentrations and triggers apoptosis, we also aimed to assess levels of M30, M65, and active caspase-3, associated with apoptosis, and to examine the relation between these parameters and S100-tau in patients with ASD. Additionally, we also intended to examine levels of NSE, reported at high levels in patients with neonatal autism [30], in our patient group and to examine its relations with S100B, tau and apoptosis. There is also still no recognized biomarker for the early diagnosis of ASD. The purpose of this study was to examine the relation between neuronal, axonal, and glial cell injury markers and autism, and to assess whether these parameters can be used as biomarkers in the diagnosis of ASD.

METHODS

Study Population

Forty-three children aged 3–12 years (30 boys, 13 girls) presenting to our outpatient clinic between March 9, 2017 and March 29, 2018, diagnosed with ASD on the basis of Diagnostic and Statistical Manual of Mental Disorders, 5th edition [37], and meeting the inclusion criteria, were included as the study group. During this time, 65 children with ASD presented to our polyclinic for the first time, but 10 exhibiting developmental regression, 9 using medication, and three diagnosed with epilepsy were excluded from our study.

The Childhood Autism Rating Scale (CARS), the validity and reliability of whose Turkish-language version has previously been confirmed [38,39], was used to evaluate the severity of ASD symptoms. CARS consists of 15 subsections, and has a minimum possible score of 15 and a maximum possible score of 60. Accordingly, children scoring 30–36.5 are mildly-moderately autistic, and those scoring 37–60 are severely autistic [38]. The socio-demographic data form elicited information concerning first name and surname, age, sex, and parents' ages. Patients were evaluated by pediatric neurology and pediatric metabolism specialists in terms of secondary autism, and magnetic resonance imaging of the brain and

electroencephalography were also performed in the context of the study.

Exclusion criteria in the patient group were any comorbid psychological disorder during evaluation (except intellectual disability), use of any medication (including vitamins and antioxidant medication) within the previous two weeks, presence of acute (such as infection (body temperature was assessed during history-taking and examination, and complete blood counts were performed) or chronic medical disease, and history of alcohol or substance use during pregnancy of mother.

The control group consisted of 41 healthy age- and sex-matched children (10 female, 31 male) admitted to the Pediatrics Department, Healthy Child Follow-up Clinic during the study period. These children in the control group underwent physical and psychiatric examinations. Children with no psychiatric and neurological, metabolic, or endocrine disease or infections, no known genetic syndromes or allergic reactions, and not using any medications were enrolled. History of maternal alcohol and substance use during pregnancy was also adopted as an exclusion criterion.

Ethics

The study was approved by the Harran University Medical Faculty Ethics Committee (session No. 03, decision No. 31, dated 09-03-2017), and was also supported by the Scientific Research Coordination Office (date 08-12-2017, protocol No. 17187).

All parents or legal guardians of the participating children gave written informed consent prior to inclusion in the study, in accordance with the Declaration of Helsinki as amended by the World Medical Association Declaration of Helsinki.

Blood Specimen Collection and Biochemical Analysis

Five milliliters of blood was collected from the antecubital vein following at least 8-hours fasting from the children comprising the patient and control groups, and was placed into gel tubes. These were immediately centrifuged at 4,000 rpm for 10 minutes at 4°C. The serum part was separated and divided into two parts, which were then stored in Eppendorf tubes at –80°C until analysis. S100B, NSE, tau, M30, M65 and active caspase-3 levels in sera were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method in the biochemistry labo-

ratory. Specimens were read at 450 nm on a microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, MA, USA). Human NSE was studied using a Fine Test ELISA kit (Catalogue No. [CN]: EH0370). NSE levels were expressed as ng/ml. Human S100B was studied using a Fine Test ELISA kit (CN. EH0543). S100B levels were expressed as pg/ml. Human tau was also measured using a Fine Test ELISA kit (CN. EH2030). Tau levels were expressed as pg/ml. Human M30 (Cytokeratin 18-M30) was studied using a Fine Test ELISA kit (CN. EH4188), with the results being expressed as mIU/ml.

Human M65 (Cytokeratin 18-M65) was measured using a Fine Test ELISA kit (CN. EH2820), and levels were expressed as ng/ml. Finally, human active caspase-3 was studied using a SunRed ELISA kit (CN. 201-12-0970A), and levels were expressed as pg/ml.

Statistical Analysis

SPSS 23.0 software (IBM Co., Armonk, NY, USA) was used for statistical analysis, and categorical variables were analyzed using Pearson's chi-square test. Normality of distribution was tested using the Shapiro–Wilk normality test. Since data were not normally distributed, non-parametric tests were applied. The Mann–Whitney *U* test was used for continuous and two-way group comparisons, and data were expressed as median values (interquartile range). Spearman's correlation analysis was applied to determine correlations. The receiver operator characteristics (ROC) curve method was employed to evaluate the accuracy of S100B, NSE, tau, M30, M65 and active caspase-3 in diagnosing ASD. The ROC was plot-

ted to determine cut-off points. *p* values < 0.05 were regarded as statistically significant.

RESULTS

Median ages were six years in the patient group (30 male, 13 female) and seven years in the control group (31 male, 10 female). No statistically significant difference was determined between the groups in terms of age, gender, parents' ages ($p > 0.05$) (Table 1). CARS scores were mild-moderate in 12 (27.9%) patients and severe in 31 (72.1%). The median CARS score in the ASD group was 41 (interquartile range: 8.5). Serum S100B, tau, NSE, active caspase-3, M30, and M65 levels were significantly higher in the patient group than in the control group ($p < 0.001$, $p = 0.002$, $p = 0.002$, $p = 0.005$, $p < 0.001$, and $p = 0.004$, respectively) (Table 1).

Spearman's rho correlation analysis determined no significant relation between CARS score and S100B, tau, NSE, active caspase-3, M30, and M65 ($p > 0.05$). Significant inverse correlation was determined between age of the patients and M65 levels ($r = -0.455$, $p = 0.002$), but none with other parameters ($p > 0.05$). Significant positive correlation was observed between tau levels and NSE and S100B levels ($r = 0.597$, $p < 0.001$; and $r = 0.433$, $p = 0.004$, respectively). Significant positive correlation was observed between NSE levels and M65 and S100B levels ($r = 0.445$, $p = 0.003$; and $r = 0.561$, $p < 0.001$, respectively). M65 levels were significantly positively correlated with S100B levels ($r = 0.507$, $p = 0.001$). Significant positive correlation was also determined be-

Table 1. Sociodemographic characteristics and S100B, tau, NSE, active caspase-3, M30, and M65 serum levels of the autism spectrum disorder and control groups

Parameters	Patients (n = 43)	Controls (n = 41)	<i>p</i> value	χ^2	Z
Sex, F/M ^a	13 (30.2)/30 (69.8)	10 (24.4)/31 (75.6)	0.548	0.360	
Age (yr) ^b	6 (3)	7 (3)	0.217		-1.235
Mother's age (yr) ^b	34 (11)	32 (8)	0.713		-0.368
Father's age (yr) ^b	37 (11)	37 (6)	0.527		-0.633
S100B (pg/ml) ^b	49.13 (2.12)	24.33 (32.64)	< 0.001		-5.979
Tau (pg/ml) ^b	131.4 (183.2)	57.5 (47)	0.002		-5.979
NSE (ng/ml) ^b	3.04 (1.15)	2.65 (0.42)	0.002		-3.043
Active caspase 3 (pg/ml) ^b	543.2 (403.5)	415 (225.5)	0.005		-2.837
M30 (mIU/ml) ^b	19.37 (8.36)	16.22 (1.21)	< 0.001		-4.484
M65 (ng/ml) ^b	0.30 (1.55)	0.20 (0.11)	0.004		-2.854

Values are presented as median (interquartile range).

NSE, neuron-specific enolase; S100B, serum 100 beta protein; F, female; M, male.

^aChi-square *p* value, ^bMann–Whitney *U* *p* value.

tween S100B and M30 levels ($r = 0.489$, $p = 0.001$).

ROC analysis was performed to determine cut-off values for the study parameters for the prediction of ASD patients. Areas under the curve (AUC) were calculated, and cut-off levels were determined as demonstrated in Figure 1. The cut-off value of parameters including tau, NSE, M65, S100B, M30 and active caspase-3 were 72.55 pg/ml (sensitivity: 69.8%, specificity: 70.7%, AUC: 0.697, $p = 0.002$), 2.83 ng/ml (sensitivity: 60.5%, specificity: 78%, AUC: 0.693, $p = 0.002$), 0.215 ng/ml (sensitivity: 69.8%, specificity: 65.9%, AUC: 0.681, $p = 0.004$), 48.085 pg/ml (sensitivity: 74.4%, specificity: 80.5%, AUC: 0.879, $p < 0.001$), 17.41 mIU/ml (sensitivity: 69.8%, specificity: 80.5%, AUC: 0.784, $p < 0.001$), and 456.4 pg/ml (sensitivity: 65.1%, specificity: 65.9%, AUC: 0.680, $p = 0.005$), respectively. High S100B values indicated ASD (Fig. 1).

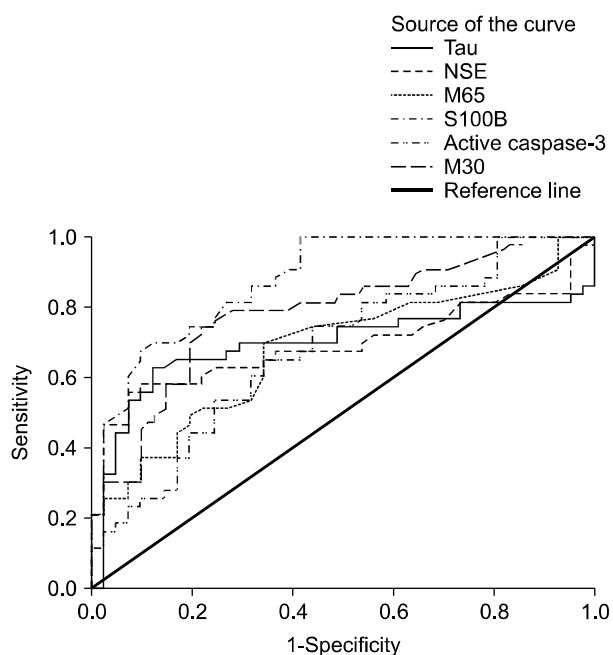


Fig. 1. Receiver operator characteristics (ROC) curves of active caspase 3, serum 100 beta protein (S100B), tau, M30, M65, neuron-specific enolase (NSE) for the prediction of autism spectrum disorders.

S100B ROC curve: area under the curve (AUC) was determined as 0.879 for S100B with a cut-off value of 48.085. Tau ROC curve: AUC was determined as 0.697 for tau with a cut-off value of 72.55. NSE ROC curve: AUC was determined as 0.693 for NSE with a cut-off value of 2.83. M65 ROC curve: AUC was determined as 0.681 for M65 with a cut-off value of 0.215. M30 ROC curve: AUC was determined as 0.784 for M30 with a cut-off value of 17.41. Active caspase 3 ROC curve: AUC was determined as 0.680 for active caspase 3 with a cut-off value of 456.4.

DISCUSSION

This study evaluated serum S100B, tau, NSE, active caspase-3, M30, and M65 levels in children with ASD, together with their sensitivity and specificity. The main finding of this study indicated an increase in serum S100B, tau, NSE, active caspase-3, M30, and M65 levels in children with ASD compared to the control subjects. In addition, the increased S100B level exhibited 80.5% sensitivity and 74.4% specificity according to ROC analysis. S100B has been reported to be neuroprotective at low concentrations and to play a role in the regeneration of injured neurons [17,18], although at high concentrations it increases the production of reactive oxygen species in neurons and mediates the death of astrocytes and neurons [19]. High blood S100B levels have even been linked to brain damage, and have been described as a predictor of poor prognosis by themselves in traumatic brain injury [40]. Additionally, high serum S100B levels have been determined in schizophrenia [20] and mood disorders [21]. A study of the identification of risk genes for ASD through copy number variation analysis in Austrian families identified S100B as one of the genes potentially associated with ASD [41]. In agreement with our own research, two recent studies showed higher serum S100B levels in children with ASD compared to control subjects [22,23]. Al-Ayadhi and Mostafa [22] determined positive correlation between serum S100B and severity of ASD, while Shaker *et al.* [23] observed no correlation, in agreement with our own study. Another study reported a high plasma S100B concentration in children with ASD [24], as well as positive correlation between both S100B levels and tumor necrosis factor-alpha and severity of ASD. A decreased serum glutathione (GSH)/oxidized glutathione (GSSG) redox ratio has also been shown in patients with ASD [42]. Increased peripheral oxidative stress and malondialdehyde (lipid peroxidation marker) levels [43,44] have also been reported in children with ASD. The fact that at high concentrations S100B increases reactive oxygen species [19], and the high serum S100B values consistent with our own study [22,23], suggest that S100B may be associated with the pathogenesis of ASD.

S100B exhibited positive correlation with tau, M30 and M65 levels in the present study. Under healthy conditions, S100B modulates the cell cycle (such as proliferation, differentiation, and apoptosis) in the central

nervous system, as well as interacting with tau to promote neurite outgrowth [45,46]. However, increased S100B levels lead to tau hyperphosphorylation, resulting in the formation of neurofibrillary tau tangles [47]. Additionally, considering the role of S100B in apoptosis, the positive correlation with M65 and M30, apoptotic cell products, in our patient group, was another important finding suggesting that S100B may be involved in the pathogenesis of ASD.

Kadak *et al.* [12] determined lower serum α -Synuclein and tau protein levels in children with ASD than in a control group. In contrast, in the present study, serum tau levels were higher in the ASD group than in the controls. Positive correlation was also determined between tau and S100B and NSE. Microtubules play a vital role in brain development, dendritic spine plasticity, myelination, synaptic formation, and the migration of developing neurons to their destinations [13]. The phosphorylated:unphosphorylated tau ratio in the brain is in balance. One study reported alterations in the phosphorylation pattern of different tau isoforms in the hippocampus, prefrontal cortex and cerebellum of the autistic-like mice strain C58/J, suggesting that alterations in tau regulation vary between different regions of the brain [11]. The decrease in the phosphorylated:unphosphorylated tau ratio in the hippocampus in that previous study supports the idea of abnormal microtubule depolymerization [11]. Moreover, since risk gene proteins associated with ASD are found in synaptic mechanisms [9], and different tau levels have been reported in patients with ASD and controls, tau may also be an important factor in the pathogenesis of ASD.

NSE is one of the important peripheral blood markers of neuronal injury [48]. One study reported hypermethylation of the enolase gene (*ENO2*) in 14.5% (number: 19/131) in patients with ASD, together with 70% less *ENO2* RNA expression compared to a control group [29]. Another previous study reported significantly higher neonatal levels of NSE in ASD patients compared to healthy controls [30]. Consistent with that study, NSE levels were also higher in our ASD group. We also determined positive correlation between NSE and tau and M65 levels. The presence of positive correlation between tau, which rises in case of axonal injury, and NSE, which rises in the event of neuronal injury, suggested that neuronal and axonal injury may play an important role in the pathogenesis of ASD.

Caspases are essential proteins with a key function in the apoptotic process. Caspase-3 is the most important caspase in the effector phase of apoptosis [49]. Levels of the apoptotic marker M30 and of the total cell death marker M65 increase with caspase activation [32]. Impairment of balance in apoptosis plays an important role in the pathogenesis of several major diseases, such as Alzheimer's, parkinson's and autoimmune diseases [33]. Studies have also investigated the role of apoptosis in ASD with central and peripheral sampling [10,35,50-52].

Studies of postmortem brain tissue in patients with ASD have reported that Bcl-2 decreased by 34%–51% in the autistic cerebellum [50], 38% in the autistic superior frontal cortex, and 36% in the autistic cerebellum [52]. Another study determined low Bcl-2 levels and high expression of the Bcl-2 regulator p53 in Purkinje cells and granule cells [35]. Studies have reported 130%, 67.5%, and 38% increases in p53 levels in the autistic parietal, superior frontal, and cerebellar cortices, respectively, while the Bcl-2/P53 ratio decreased by 64% in the autistic brain compared to control subjects [50]. Increased cathepsin D, associated with tau cleavage, and also caspase-3 have also been shown in the autistic cerebellum [10]. In addition, a review of postmortem studies of the brains of autistic patients reported cerebellar Purkinje cell atrophy, a decrease in numbers, and granule cell loss [36]. Pathological activation of apoptosis may therefore be associated with neuroanatomical changes and ASD. Another study determined lower plasma caspase-3 in children with ASD compared to control subjects [53]. The low plasma caspase-3 level in that study may have derived from the conversion of caspase-3 into active caspase-3. Indeed, the other study showed up-regulation and activation of several caspases, together with increases in levels of caspase-3, -7, and -12 in peripheral blood mononuclear cells of children with ASD [54]. Similarly to the increased apoptosis in children with ASD described above, we also observed higher active caspase-3, M30 and M65 levels in our ASD group compared to the control group. Evaluation of this increase in serum active caspase-3 and M30 levels in our study together with the decrease in Bcl-2 [50] and the increase in p53 [35] shown in the brain tissue of individuals with ASD suggested that apoptosis increases in children with ASD. In addition, evaluation of the increase in M65 (the total cell death marker) levels in our study together with the atrophy in

and loss of cerebellar cells and the low number of those cells [36] suggested that apoptosis and necrosis increase in neuronal cells in children with ASD. Indeed, significant inverse correlation between age and serum M65 levels in patients diagnosed with ASD suggested that apoptosis and necrosis in neuron and glial cells was more intense in younger children. Additionally, M30 and M65 exhibiting positive correlation with S100B, NSE, and M65 suggests an association with the apoptotic process in neuronal and astrocyte cells, and that injury in these cells may be associated with the pathogenesis of ASD.

Another important finding of this report was elicited by the ROC curve analysis applied to assess the diagnostic value of increased S100B, tau, NSE, active caspase-3, M30, and M65 parameters. Although recent studies have investigated pro-oxidant and antioxidant markers of potential diagnostic value in ASD, there is still no generally accepted biomarker for diagnosing and determining the severity of ASD [2,3]. The majority of the markers evaluated in the present study have not previously been investigated as diagnostic biomarkers in children with ASD. Only one study has evaluated a low plasma caspase level (AUC: 0.968) as a diagnostic biomarker in children with ASD [53]. In contrast to that study, we assessed active caspase-3 levels and determined significant elevation in children with ASD. The high active caspase-3 level, one finding from our study, and the low caspase-3 level shown in the previous study [53] suggest that apoptosis increases in children with ASD. ROC analysis applied in order to evaluate active caspase-3 in children with ASD (sensitivity: 65.1%, specificity: 65.9%, AUC: 0.680), revealed no diagnostic value. ROC analysis also revealed no diagnostic value in terms of ASD for tau (sensitivity: 69.8%, specificity: 70.7%, AUC: 0.697), NSE (sensitivity: 60.5%, specificity: 78%, AUC: 0.693), M65 (sensitivity: 69.8%, specificity: 65.9%, AUC: 0.681) or M30 (sensitivity: 69.8%, specificity: 80.5%, AUC: 0.784). In addition, ROC analysis identified S100B (sensitivity: 74.4%, specificity: 80.5%, AUC: 0.879) as having good diagnostic value in children with ASD.

Limitations of the present study include the low sample number, and the fact that parameters were studied in serum, not in cerebrospinal fluid. The diagnostic value of these parameters in children with ASD should now be evaluated in further studies with more extensive samples.

In conclusion, our study indicated an increase in serum

S100B, tau, NSE, active caspase-3, M30, and M65 levels in children with ASD. In addition, positive correlation was determined between S100B and tau, M30, and M65 levels, between tau and NSE, and between NSE and M65 levels. Inverse correlation was determined between patient age and M65. These findings suggest the presence of neuronal, axonal, and glial cell injury in children diagnosed with ASD, and that apoptosis and necrosis increase and may be more intense in younger children in particular. This also supports the importance of early diagnosis and intervention in autism. In addition, our findings suggest that S100B may be a promising, potential biomarker for ASD and worthy of further study.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conceptualization: Hamza Ayaydın, İsmail Koyuncu. Data acquisition: Hamza Ayaydın, Şermin Bilgen Ulgar, İsmail Akaltun, Adnan Kirit. Formal analysis: Hamza Ayaydın, Adnan Kirit, İsmail Koyuncu, Hakim Çelik, Şermin Bilgen Ulgar, İsmail Akaltun. Supervision: Hamza Ayaydın. Writing—original draft: Hamza Ayaydın, İsmail Koyuncu, Hakim Çelik. Writing—review & editing: Hamza Ayaydın, İsmail Koyuncu, Adnan Kirit.

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