International Journal of Developmental Neuroscience 61 (2017) 86–91

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Research Papers

Evaluation of serum Neuron-specific enolase, S100B, myelin basic protein and glial fibrilliary acidic protein as brain specific proteins in children with autism spectrum disorder

Erman Esnafoglu[a,*], Sema Nur Ayyıldız[b], Selma Cırrık[c], Emine Yurdakul Erturk[d], Abdullah Erdil[d], Abdullah Daglı[d], Tevfik Noyan[b]

[a] Department of Child and Adolescent Psychiatry, Training and Research Hospital, Faculty of Medicine, Ordu University, Ordu, Turkey
[b] Department of Biochemistry, Training and Research Hospital, Faculty of Medicine, Ordu University, Ordu, Turkey
[c] Department of Medical Physiology, Faculty of Medicine, Ordu University, Ordu, Turkey
[d] Department of Pediatry, Training and Research Hospital, Faculty of Medicine, Ordu University, Ordu, Turkey

ARTICLE INFO

Keywords:
Autism spectrum disorder
Brain specific proteins
Neuron specific enolase
Glial fibrillary acidic protein
Myelin basic protein
S100B

ABSTRACT

Objective: Brain specific-proteins are not found in other tissues and measurement non-invasively in the blood may identify structurally and functionally damaged brain regions and identify the severity and prognosis of neuropsychiatric diseases. For this reason, we aimed to evaluate serum brain-specific protein values as brain damage markers in children with autism spectrum disorder (ASD).

Method: 35 children with ASD and 31 healthy subjects were included in the study. Sociodemographic form and Childhood Autism Rating Scale (CARS) were applied to each subject. Serum neuron specific enolase (NSE), S100B, Myelin basic protein (MBP) and Glial fibrillary acidic protein (GFAP) values were measured with ELISA.

Results: There was no significant difference between the two groups for NSE, MBP and S100 B values ($p = 0.242$; $p = 0.768$; $p = 0.672$, respectively). However, GFAP values in the patient group were statistically significantly higher (mean ± SD: 0.463 ± 0.392 ng/ml) than in the healthy control group (mean ± SD: 0.256 ± 0.111 ng/ml) ($p < 0.001$). In addition, there was a significant positive correlation between serum GFAP values and CARS score in all subjects and in the patient group ($r = 0.599$; $p < 0.001$ and $r = 0.380$; $p = 0.024$, respectively).

Conclusions: While serum NSE, MBP, and S100 B values cannot be considered as biomarkers for ASD, GFAP may be a biomarker and is suggested as a possible indicator of autism severity.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by typical social communication, social interaction and repetitive/stereotypical behavior with high rates of inheritance (APA, 2013; Lai et al., 2014). Epidemiological studies have estimated the prevalence as 1.1% (Baron-Cohen et al., 2009). Another epidemiological study in South Korea identified the prevalence of ASD according to DSM-5 as 2.2% (Kim et al., 2014). Assessed as a multifactorial disorder comprising the interactions of neurological, immunological, environmental and genetic factors, the cause of autistic disorder is still not definitely known (Ashwood et al., 2006).

Biomarkers are accepted as molecules, enzymes or proteins measured in body fluids, or tissues and cells. They are used as diagnostic and prognostic values reflecting the underlying situation or disease. Biomarkers are accurate, sensitive, specific, reliable and have high predictive value (Mayeux, 2004; Cook, 2008). It is very important to identify biomarkers for ASD because children with ASD find it difficult to communicate and cannot easily express their symptoms (Sokolowska et al., 2015). Additionally, there is no objective method to identify the disorder. In place of this, diagnosis is made based on observation of behavior in a very subjective manner (Ratajczak, 2011). Brain specific proteins are not found in other tissues and measurement non-invasively in blood may identify structurally and functionally damaged brain regions and identify the severity and prognosis of neuropsychiatric diseases (Lamers et al., 2003).

The S100 B protein family regulates protein phosphorylation, transcription factors and enzyme activities, and affects continuation of Ca^{+2} homeostasis, cell growth and differentiation, inflammatory response and cell skeleton (Donato, 2003; Sen and Belli, 2007). S100 B is

* Corresponding author at: Ordu Universitesi Boztepe Egitim ve Arastırma Hastanesi, Bucak Mah., Altınordu/Ordu, 52200, Turkey.
E-mail address: ermanesnafoglu@yahoo.com.tr (E. Esnafoglu).

http://dx.doi.org/10.1016/j.ijdevneu.2017.06.011
Received 29 March 2017; Received in revised form 12 June 2017; Accepted 30 June 2017

localized to the astro-oligodendrocytes and is released from them. S100 B at nanomolar concentrations behaves like a growth and differentiation factor for neurons and glials; at higher (micromolar) levels it may have damaging effects and increase expression of proinflammatory cytokines initiating apoptosis (Rothermundt et al., 2003). S100 B in peripheral circulation may be identified as a marker of breakdown of the blood-brain barrier (BBB) (Marchi et al., 2004). S100 B is commonly considered a marker of glia activation in response to brain damage. Additionally the neurotrophic effect of S100 B is not known. S100 B plays a role in brain development and may contribute to developmental pathogenesis in psychiatric diseases (Manev and Manev, 2001). Increased S100 B levels in peripheral blood are reported to be found with some neurological and psychiatric diseases. Serum S100 B levels increase in schizophrenic patients, it accompanies negative symptoms and levels have been shown to fall after treatment (Rothermundt et al., 2001). Other studies of schizophrenic patients found increased S100 B as a peripheral marker of brain damage (Aleksovska et al., 2014; Schmitt et al., 2005; Pedersen et al., 2008; Steiner et al., 2006). In children with cerebral palsy and developmental retardation, S100 B overexpression has been identified (Park et al., 2004). Studies have identified increased S100 B levels in mood disorders and especially depression (Schroeter et al., 2013). Additionally S100 B increases in parallel with suicidal thoughts in adolescent patients and it is proposed that it may be a potential marker of suicidality (Falcone et al., 2010). A few studies of ASD children have found increased serum S100 B levels (Al-Ayadhi and Mostafa, 2012; Shaker et al., 2016).

In addition to S100B, neuron specific enolase (NSE) is a specific serum marker of neuronal injury because NSE is primarily localized to neuron cytoplasm with increases in cerebrospinal fluid and blood indicating structural damage to neuronal cells (Kaiser et al., 1989; Marangos and Schmechel, 1987; Royds et al., 1981). Ensolases are dimers formed of a combination of three separate subunits (α, β and γ). The NSE yy-dimer has 78 kDA molecular weight and is expressed by neurons in the central nervous system and by neuroendocrine cells in the periphery (Matyar et al., 2016). The yy-dimer of NSE occurs during neurogenesis and increases during neuronal differentiation; this makes it a good marker of neuronal differentiation and maturation (Kaiser et al., 1989). Additionally it is reported that NSE has neurotrophic and neuroprotective effects (Hattori et al., 1995; Hafner et al., 2013). NSE controls the PI3 K/Akt and MAPK/ERK signal pathways in neuronal survival, differentiation and neurite regeneration (Hafner et al., 2012). Enolase is a basic catabolic enzyme transforming 2-phosphoglycerate to phosphenolpyruvate on the glycolytic pathway synthesizing ATP (Kawata et al., 2016). NSE is an important molecule for maintenance of the excitability of the neuronal membrane. Under normal conditions, there is very little NSE in body fluids. Immediately after neuronal cell damage, it begins to be released into body fluids (Hamed et al., 2016). NSE has been researched in many childhood period neurological disorders (Lima et al., 2004). NSE is helpful in the diagnosis of neurologic diseases like ischemic stroke, intracerebral hemorrhage, epilepsy, traumatic brain injury, Guillain-Barre syndrome, Alzheimer disease, delirium and Creutzfeldt-Jacob disease (Matyar et al., 2016; Isgrò et al., 2015). Additionally increase NSE levels have been identified in tumors like melanoma, seminoma, renal cell carcinoma and phaechromocytoma (Isgrò et al., 2015). A study of schizophrenia found increased S100B, with no change in NSE levels found (Steiner et al., 2006; Schroeter et al., 2009). It has been investigated in mood disorders like depression and bipolar disorder (Wiener et al., 2016; Wiener et al., 2013). To the best of our knowledge, only one study has assessed NSE levels in children with ASD. This study retrospectively compared ASD and healthy control groups in the neonatal period. Accordingly NSE was clearly high in the neonatal period in children with ASD (Lv et al., 2016).

Apart from S100 B and NSE which may show neuronal, astrocytic and oligodendrocytic damage in neuropsychiatric diseases, glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) are other potential markers (Lamers et al., 2003). Although MBP autoantibodies have been investigated in autistic children to date, serum MBP levels have not been investigated (Mostafa and A.L-Ayadhi, 2011; Mostafa and Al-Ayadhi, 2013). MBP is the basic protein part of the myelin sheath and comprises 30% of myelin (Ohta and Ohta, 2002). Normally MBP is only synthesized by oligodendrocytes and Schwann cells. During active demyelinization processes and in neurological disease with myelin damage, it increases in body fluids (Whitaker, 1998). Especially in multiple sclerosis, MBP levels in body fluids increase in proportion to severity of the disease (Ohta and Ohta, 2002).

Glial fibrillary acidic protein (GFAP) is a relatively non-soluble acidic cytoskeletal protein and is a basic intermediate filament found in astrocytes in the human brain (Petzold, 2015). GFAP plays an important role in astroglia cell activation (astrogliosis) observed in situations like central nervous system injury and neurodegeneration. As a result GFAP is accepted as a marker with high specificity for brain damage (Yang and Wang, 2015; Hol and Pekny, 2015). With this aim, S100B, NSE, GFAP and MBP markers have been studied in serum and cerebrospinal fluid (CSF) to determine astrocytic, oligodendrocytic and neuronal damage in schizophrenia (Steiner et al., 2006). Another study including serum proteins used S100B, GFAP and MBP and recommended them for diagnosis and clinical assessment in schizophrenia (Xiong et al., 2014). In children with infantile autism, GFAP in CSF was found to be higher compared to normal children in the same age interval (Rosengren et al., 1992). Again GFAP in CSF was found to be nearly three times higher in autistic children compared to a normal group. This finding indicates gliosis and non-specific brain damage in autism (Ahlsén et al., 1993). A postmortem study identified GFAP was significantly increased in the frontal, parietal and cerebellar cortices in autistic children. This confirms microglial and astroglial activation in autism. Increased GFAP levels in autistic brains indicate gliosis, reactive injury and disrupted neuronal migration processes (Laurence and Fatemi, 2005). NSE, S100 B and GFAP are secreted by brain cells and probably pass a disrupted blood brain barrier to join systemic circulation (Lamers et al., 2003).

In this study of ASD patients, values in circulation of NSE, S100B, MBP and GFAP were researched to assess whether they are biomarkers for neuronal and glial injury.

2. Methods

Thirty-five ASD patients (mean age ± SD 7.6 ± 3.62; 9 female, 26 male) and 31 healthy control subjects (mean age ± SD 6.85 ± 3.16; 6 female, 25 male) were recruited from Ordu University Faculty of Medicine Training and Research Hospital Child and Adolescent Psychiatry outpatient clinic. The ethnicity of all subjects was Turkish. The family of each participant was informed about the study and then written consent was obtained. The study was approved by Ordu University Faculty of Medicine ethics committee. All participants completed a sociodemographic form and the Childhood Autism Rating Scale (CARS). As a result of detailed clinical observation and family interviews, 35 ASD children were diagnosed according to criteria from Diagnostic and Statistical Manual of Mental Disorders-V (APA, 2013). General examination was performed for each participant by expert pediatricians. When creating the patient and control groups, those with medical treatment within the last 6 months, chronic diseases, tumors, epilepsy and neurological deficits were excluded. The healthy control group was created from those attending the child psychiatric clinic for consultation on minor problems. All subjects had routine laboratory tests applied. 5 ml of blood were collected by venipuncture from the antecubital vein, in a vacutainer tube without anticoagulant, in the morning between 09:00 and 11:00 just before breakfast. After collection, the total blood was immediately centrifuged at 4000*g* for 10 min. Following centrifugation, the serum was transferred to an eppendorf tube and stored −80 °C prior to measurements.