Effects of Non-Pharmacological Interventions on Serum Levels of S100B, NT3 and BDNF in Children with Autism Spectrum Disorder

Non-Farmakolojik Girişimlerin Otizm Spektrum Bozukluğu Olan Çocuklarda Serum S100B, NT3, ve BDNF Düzeylerine Etkileri

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ABSTRACT

Objective: The aim of this study was to examine the effects of non-pharmacological interventions (NPI) on the serum levels of S100B, NT3 and BDNF in patients with autism spectrum disorder (ASD).
Methods: All participants were evaluated by to two child and adolescent psychiatrist per DSM-5 criteria. We evaluated 43 cases with ASD and 26 inviduals as a healthy control group between the ages of 0-6 that presented to the outpatient clinic between March 2014 - May 2015.
Results: S100B level was found to be higher in ASD compared to Control group(C) and ASD+NPI. There was no difference between C and ASD+NPI. NT3 was lower in ASD and C than ASD+NPI. Although BDNF was not different between groups, a significant positive correlation between NT3 and BDNF was found in ASD+NPI.
Conclusion: NPI appears to reverse the increased level of S100B and produce alterations in BDNF and NT3 in ASD patients.

Keywords: Autism spectrum disorder, S100B, NT3, BDNF, non-pharmacological interventions

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ÖZET

Amaç: Bu çalışmanın amacı otizm spektrum bozukluğu (OSB) olan hastalarda uygulanan non-farmakolojik tedavinin (NFT) serum S100B, NT3 ve BDNF düzeylerinde oluşabilecek olası değişiklikleri incelemektir.
Yöntem: Serum S100B, NT3 ve BDNF düzeyleri ELISA yöntemi ile ölçülmüştür.
Bulgular: S100B düzeylerinin OSB'de OSB+NFT grubundan anlamlı olarak yüksek bulunmuştur. Kontrol, OSB ve OSB+NFT grupları arasında ise fark yoktur. BDNF'nin serum düzeyi gruplar arasında farklı olmamasına rağmen OSB+NFT gruplarında NT3 ile arasında anlamlı bir korelasyon mevcuttur.
Sonuç: Bu bulgular NFT uygulamasının OSB grubunda artmış olan S100B düzeylerini geri çevirebileceğini ve BDNF ile NT3 düzeylerinde değişiklikler oluşturabileceğini telkin etmektedir.

Anahtar Sözcükler: Otistik spektrum bozukluğu, otizm, S100B, NT3, BDNF, non-farmakolojik tedavi

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INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impairments in communication, reciprocal social interaction, presence of restricted and repetitive patterns of behavior (1). Although numerous studies showed that genetic, environmental, neurological and immunological factors are implicated, the etiopathogenesis of ASD remains poorly understood (2). Recent studies have focused on the contribution of neuroinflammation and neurotrophic factors (3).

The major source of S100B is activated astrocytes during neuroinflammation (4). Low concentrations of S100B (nM) promote neuronal survival while high concentrations of S100B (μM) are associated with neuroinflammation and neuronal apoptosis (5). It has been suggested that S100B is a a reliable and early marker in CNS pathologies at the molecular level which cannot be detected in neuroimaging and physical examination (6). Although numerous studies indicate the contribution of neuroinflammatory changes to ASD, elevated S100B level in serum has been reported in merely two studies (7, 8).

Neurotrophic factors including BDNF, NT3 and NT4 play a crucial role in neurogenesis, neuronal migration, growth and survival of neurons, synaptogenesis and synaptic plasticity (9). Several studies show that neurotrophic factors are involved in the pathogenesis of ASD (10, 11).

NT3 has been demonstrated to contribute to the development of cerebellar purkinje cells and maturation of glutamatergic synapses (12). Consistenly, lower number of purkinje cells have been reported in patients with ASD (13). Nevertheless, there are controversial studies showing the unchanged or reduced peripheral levels of NT3 in ASD (11, 14). Additionally, it has been demonstrated that BDNF supports the growth and survival of cholinergic, serotonergic and dopaminergic neurons (15, 16). Although there are controversial reports about the how serum levels is affected, BDNF is implicated in pathophysiology of ASD (17, 18).

Studies support the efficacy of non-pharmacological interventions(NPI) for improving targeted skills or global outcomes in individuals with ASD [30]. In this framework, NPI for the patients with ASD are considered a treatment modality which is able to boost neuroplasticity (19). In favor of NPI, cognitive activity, physical exercise and sensory stimulation has been shown to lead improvement in neurobiological parameters (20-22). It is known that BDNF and NT3 levels are altered due to enviromental enrichment (EE) which corresponds to NPI in animal models (23, 24).

In the present study, the aim was to determine the NPI-induced alterations in S100B level in relation to neurotrophic factor levels in serum including BDNF and NT3. This study might contribute to both our understanding of the etiopathogenesis and the mechanisms of effects of NPI. Further studies are required to classify the different methodologies of NPI to provide the most effective outcomes.

METHODS

Study Design

This study retrospectively examined 43 children diagnosed with ASD based on DSM-5 criteria who presented to the Child and Adolescent Psychiatry Department in Gazi University Faculty of Medicine. ASD patients were divided into two groups; ASD included those diagnosed with ASD, ASD+NPI included patients who underwent NPI. NPI is an individualized educational program for children with ASD in handicapped areas and includes behavioral interventions. In this study, ASD+NPI group received “Pervasive Developmental Disorders Supportive Training Program” in Turkey by educated teachers, 8-10 hours in a month. Individuals with at least 6 months of this training program were included in the study (25). There were 26 children in ASD group, 17 in ASD+NPI, and 26 in the control group. Blood samples were collected from all participants, after centrifugation the serum was stored at −80 °C.

Informed consent was obtained from all individuals included in the study. Ethical approval for the study was taken from Gazi University Medical School Research Ethics Committee (11 February 2014, 25901600-713) and the study has been performed in accordance with the ethical standards laid out in the 1964 Declaration of Helsinki and its later amendments.

Case Group

The psychiatric assessment was based on clinical history taken from caregivers and neuropsychiatric assessment. All children meeting the diagnostic criteria for autism spectrum disorder in the DSM-5 were included in this study. A comprehensive diagnostic approach including genetic, metabolic, neurological and psychiatric examination was applied to all participants. After the examinations, children with chronic medical comorbid condition (neurological, metabolic, allergic, inflammatory and autoimmune diseases etc.), or those who used any pharmacological treatment were excluded.

Control Group

The patients who were admitted to Pediatrics Health Department and didn’t have any neurological, allergic, immunological, psychiatric disdorders or pharmacological treatment in the last six months were included in the control group.

Measures

Childhood Autism Rating Scale (CARS): The Childhood Autism Rating Scale which rates the child on a scale from 1 (no signs of autism) to 4 (severe symptoms) in a 15-item Likert-type behavioral rating scale used to detect and count the symptoms of autism and to discriminate them from other neurodevelopmental disorders. Good evidence of reliability and validity has been reported in Turkish children with autism (26).

Measurement of Serum S100B, BDNF, NT3 levels

A 2-3 cc blood samples were taken from all participants and then rapidly sent to the laboratory at room temperature within 30 minutes. After centrifuging (3000 g/7 min) the serum was pipetted into eppendorf tube. Until the day of analysis, samples were stored at -80 ° C freezer. Diametra S100B (Lot: 20140728), RayBio Human BDNF (lot: 3610116113) and NT-3 Human ELISA (lot: 19110128113) kits were used to determine the level of Serum S100B, BDNF, NT3.

Statistical Analysis

Research data was analyzed with "Statistical Package for Social Sciences for Windows 22.0 (SPSS Inc, Chicago, IL)". Descriptive statistics are categorized as median (minimum-maximum), frequency distribution and percentage. Pearson's chi-square was test used for the evaluation of categorical variables. Compliance with the normal distribution of variables was evaluated using visual (histograms and probability plots) and analytical methods (Shapiro-Wilk test). S100B, BDNF and NT3 variables didn’t fit to normal distribution, so Mann-Whitney U test was used for statistical significance between the two independent groups, Kruskal-Wallis test was used for statistical analyzes between the three independent groups. When significant difference was detected between three independent groups, post-hoc Bonferroni correction was applied to determine the source of the difference. The relationship between variables was assessed by Spearman correlation analysis. Statistical significance level was adopted as $p<0.05$.

RESULTS

Sixty-nine children (59 boys, 10 girls) were evaluated within the scope of this research. The average age of participants was 44.32 ± 9.63 (min: 25-max: 65) months.In this study, 26 of the children (37.7% of the control group) were healthy and called as "control group”. 17 of the children (24.6%) with ASD who had been treated with NPI were called as ‘ASD+NPI’ group. 26 of the children (37.7%) with ASD who were not part of any pharmacological or NPI were called as “ASD”. Specified groups were considered as working groups. The distribution of blood S100B BDNF and NT3 levels in the study group, are shown in Table 1.

Table 1. Distribution of serum S100B, BDNF and NT3 levels in study groups

	ASD (n=26)	**ASD**+NPI **(n=17)**	**Control (n=26)**	**p***
	Mean (min-max)	Mean (min-max)	Mean (min-max)	
S100B (ng/L)	1436 (13-5339)[bc]	400 (52-2150)	226 (5-3000)	**<0,001**
BDNF (pg/ml)	3571 (1350-6764)	3508 (632-12178)	2894 (294-6806)	0,271
NT3 (pg/ml)	97 (41-209)	153 (69-209)[ac]	97 (41-209)	**0,014**

%: Column Percentage; mean±SD Mean ± Standard Deviation *Kruskal Wallis Test
aIt was statistically significant difference with "Treated ASD" group in Post-hoc pairwise comparisons (p<0,017)
bIt was statistically significant difference with "Untreated ASD" group in Post-hoc pairwise comparisons (p<0,017)
cIt was statistically significant difference with "Control"group in Post-hoc pairwise comparisons (p<0,017)

The groups were found to be statistically significant in blood NT3 and S100B values (p <0.05). In post-hoc pairwise comparisons, there was significant differences in S100B and NT3 levels between ASD and ASD+NPI groups.

While the patients in ASD group had significantly higher serum S100B levels than the patients in ASD+NPI group (p = 0.003) and control group (p <0.001), the patients in ASD+NPI group had significantly higher serum NT3 levels than ASD group (p = 0.008) and control group (p = 0.009) (Table 1, Figure 1). On the other hand study groups had no significant difference in serum BDNF levels (p> 0.05) (Table 1, Figure 1).

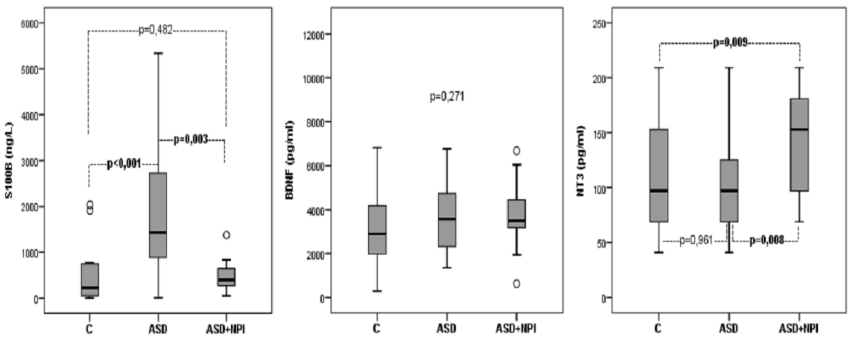

Figure 1. Distribution of serum S100B, BDNF and NT3 levels in study groups

The relationship between age, serum S100B, BDNF and NT3 levels is presented in Table 2. There was no significant correlation between age, serum S100B, BDNF and NT3 levels in ASD group (p> 0.05) (Table 2).

Positive, strong and statistically significant correlation has been found between serum BDNF and NT3 levels (r = 0.61, p = 0.009), there was no significant relationship between other variables (p> 0.05) in ASD+NPI group (Table 2, Figure 2). There was no significant correlation between age, serum S100B, BDNF and NT3 levels in control group (p> 0.05) (Table 2).

Table 2. Relationship between age, serum S100B, BDNF and NT3 levels in study groups

		Age(month)	**S100B (ng/L)**	**BDNF (pg/ml)**	**NT3 (pg/ml)**
		r	r	r	r
ASD (n=26)	**Age(month)**	1,000	-0,349	-0,128	0,205
	S100B (ng/L)	-0,349	1,000	0,073	-0,315
	BDNF (pg/ml)	-0,128	0,073	1,000	0,025
	NT3 (pg/ml)	0,205	-0,315	0,025	1,000
ASD+NPI (n=17)	**Age(month)**	1,000	0,167	0,271	-0,258
	S100B (ng/L)	0,167	1,000	0,407	0,024
	BDNF (pg/ml)	0,271	0,407	1,000	***0,614*****[**]**
	NT3 (pg/ml)	-0,258	0,024	***0,614*****[**]**	1,000
Control (n=26)	**Age(month)**	1,000	-0,263	0,274	-0,256
	S100B (ng/L)	-0,263	1,000	0,106	-0,013
	BDNF (pg/ml)	0,274	0,106	1,000	0,058
	NT3 (pg/ml)	-0,256	-0,013	0,058	1,000

r=Spearman Correlation Coefficient; *p<0,05; **p<0,01

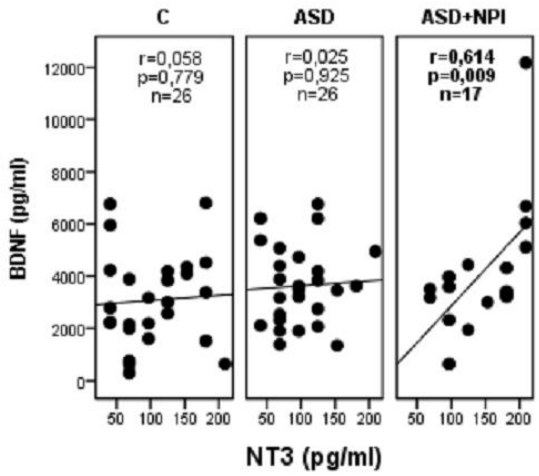

Figure 2. Relationship between age, serum S100B, BDNF and NT3 levels in study groups

In ASD and ASD+NPI groups, distribution of blood S100B, BDNF and NT3 values in accordance with severity of the ASD is presented at Table 3. In ASD group, even though there was a significant difference in S100B value based on disease severity ($p < 0.05$), there was no significant difference in BDNF and NT3 values ($p > 0.05$). Also in ASD group, S100B levels were significantly higher in severe ASD patients than mild group (Table 3). In ASD+NPI group, there was no statistically significant difference in serum S100B, BDNF, NT3 levels in term of disease severity ($p > 0.05$) (Table 3).

Table 3. Distribution of blood S100B, BDNF and NT3 values between disease severity in ASD and ASD+NPI groups

		Mild ASD		Severe ASD		p*
		n	Median (min-max)	n	Median (min-max)	
ASD	S100B (ng/L)	17	1105 (13-3187)	9	2724 (890-5339)	**0,008**
	BDNF (pg/ml)	17	3634 (1350-6764)	9	2324 (1392-5368)	0,200
	NT3 (pg/ml)	17	97 (41-209)	9	69 (41-153)	0,200
ASD+ NPI	S100B (ng/L)	12	381 (52-2150)	5	415 (186-2073)	0,721
	BDNF (pg/ml)	12	3507 (2324-12178)	5	3508 (632-5114)	0,574
	NT3 (pg/ml)	12	181 (69-209)	5	125 (69-209)	*0,383*

ASD: Autism Spectrum Disorder; *Mann-Whitney U Test

DISCUSSION

S100B is prominently expressed in astroglial cells (4). Its activity in the brain both as an intracellular and extracellular signal regülatör is of importance (5). Astrocytes release S100B into the extracellular space constitutively and it acts on neurons, astrocytes and microglia via its primary receptor named Receptor for Advanced Glycation End Products (RAGE) (27, 28). As an extracellular factor, low concentrations of S100B (nM) promotes neuronal survival and high concentrations (μM) produce harmful events such as brain inflammation and neuronal apoptosis (5). In addition, S100B stimulates microglial activation, leading to the release of interleukin-6 and interleukin 1α in pathological conditions (29). Thus activation of astrocytes and microglia is involved in neuroinflammatory processes (30). Neuroinflammation is also crucial in neuronal loss in CNS. Activation of TLRs (Toll-like receptors) expressed in microglia causes injury in neurons (31).

Elevated S100B levels in serum or cerebrospinal fluid (CSF) could reflect the astrocyte activation and blood brain barrier dysfunction resulting from acute or chronic pathologies such as schizophrenia, multiple sclerosis, Alzheimer's Disease, brain ischemia and hemorrhage. Normal S100B levels in serum/blood or in CSF fluid reliably exclude major CNS pathology (32). The major advantage of measuring the S100B in serum or CSF is that elevations of S100B level reflects the pathological alterations at the molecular level prior to gross changes detectable in neuroimaging and neurological examination (33). In addition, serum S100B level in serum reflects the blood brain barrier (BBB) permeability even in the absence of neuronal injury (34). Extracranial sources of S100B does not affect serum levels and are not compromised in the clinical settings (35).

In the present study, it was hypothesized that S100B levels might be altered in ASD in relation to neurotrophic factors such as BDNF and NT3. Serum S100B level can be a more sensitive marker than neuroimaging to evaluate brain pathologies (36). Since neuroimaging studies do not provide sufficient the diagnostic and follow up criteria, it is clear that identification of a biomarker detectable in serum might ensure numerous benefits. Consistent with previous studies, the present study shows that serum S100B level is increased in patients with ASD at the age between 29-72 months (7, 8).

Based upon the sudies mentioned above, it is most likely that elevated levels of S100B in this study reflect the presence of ongoing neuroinflammatory molecular alterations which cannot be identified by neuroimaging techniques. Consistent with this result, S100B level has been reported to be correlated with the autistic severity (7). Taken together, S100B level might be a potential biomarker in predicting the following parameters; 1) diagnosis, 2) understanding the contribution of neuroinflammatory processess to pathophysiology, 3) efficiency of treatment, 3) prognosis, 4) new treatment approaches.

Neurotrophic factors including NT3, NT4 and BDNF play a critical role in many processes in CNS including neurogenesis, synaptic plasticity, long-term potentiation and cognitive functions (37, 38). Although the aetiology of ASD remains poorly understood, it has been suggested that neurotrophic factors are involved in the pathophysiology and they might have a potential role as promising biomarker candidates (39).

It is known that NT3 plays a key role in the maturation of glutamatergic synapse development (12). Therefore, a deficiency in NT3 level would be expected to cause a deficit in activity dependent synapse and neuron elimination in ASD, leading to impairment in brain development. Consistent with this view, several studies show that serum NT3 levels are reduced in children with ASD (40).

Additionally, Purkinje cells have been reported to be lower in patients with ASD (13). Thus, it is likely that elevated level of NT3 in this study might contribute to the impact of NPI, producing a modulatory effect on glutamatergic system.

Although BDNF level increases with age in serum, BDNF in young children with ASD has been reported to be higher than adult controls (41). Conversly, decreased level of BDNF also has been shown in other studies (18). BDNF produces trophic effects on serotonergic neurons and ASD is associated with abnormal level of serotonine (42). The level of BDNF has been indicated to be three times as high in basal forebrain of patients with ASD in comparison with adults of comparable age (43). In the present study, BDNF level in serum was not found to be higher in comparison with ASD and controls. However, a significant positive correlation has been revealed between BDNF and NT3 levels in ASD+NPI group. Therefore, these results may be interpreted to suggest that even if BDNF level did not reach a statistically significant value, it indeed increased in a positively correlated manner with NT3. Hence, interventions seem to induce not only NT3 but also BDNF. Consistenly, EE has been determined to increase BDNF level and concomitantly amplified excitatory action via formation of glutamatergic synapses (44). In addition, in line with previous studies, increased BDNF level also produces beneficial effects on the influence of interventions (45).

Brain plasticity is quite sensitive to enviromental stimuli. Numerous studies report that brain undergo alterations in response to enviromental effects (44). Interventions used in patients with ASD roughly include sensory stimulation, cognitive activity and physical exercise, and these interventions have been reported to be effective at improving the outcomes (20-22). In animal models, enhances levels of sensory stimulation, cognitive activity and physical exercise, associated with experience-dependent plasticity while contributing to brain repair (46).

In the present study, interventions reversed the increased S100B level to control values in patients with ASD. Furhermore, interventions also led to an increase in NT3 level that is positively correlated with BDNF level. Taking these together, it seems feasible to speculate that increased NT3 and BDNF levels might have contributed to the reduction of S100B level. However, it is not possible to explain exactly whether these alterations in neurotrophic factors and S100B are compensatory mechanisms or intrinsic components of the emergence of the disease process. Additionally, ASD is heterogenous and comprised of various factors.

This study might provide a clue to clarify the extent to which different components of EE or interventions can be seperated and analysed according to their useful effects. The limitation of this study is that clinical evaluation following the interventions was not performed. Therefore, alterations in clinical setting corresponding to changes in these biochemical parameters are not known.

In conclusion, S100B level was found to be higher in patients with ASD than control and ASD+NPI groups. Non-pharmacologocical interventions reversed the higher S100B level to control values and NT3 level increased significantly in this group in comparison with ASD and control values. Furhermore, there was a significant positive correlation between NT3 and BDNF also in this group.

Conflict of interest

No conflict of interest was declared by the authors.

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