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Serum Levels of Brain-Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin-3, and Glial-Derived Neurotrophic Factor in Children with Specific Learning Disorder

Gökçe Sultan UNCU[1], Ali KARAYAĞMURLU[2], Tuba BİLBAY KAYNAR[3], Adile Merve BAKİ[4], Pervin VURAL[4], Nusret SOYLU[2]

[1]Department of Child and Adolescent Psychiatry, Van Training and Research Hospital, Van, Turkey
[2]Department of Child and Adolescent Psychiatry, İstanbul Faculty of Medicine, Istanbul University, İstanbul, Turkey
[3]Department of Child and Adolescent Psychiatry, Muş State Hospital, Mus, Turkey
[4]Department of Biochemistry, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey

ABSTRACT

Introduction: Specific learning disorder (SLD) is a neurodevelopmental disorder that involves complex interactions of genetic, neurobiological and environmental factors, but the definite mechanisms remain mostly unknown. The possible role of neurotrophins has been implicated in the pathophysiology of various neurodevelopmental disorders. This study aimed to investigate whether serum levels of brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3) in children with SLD deviate from those of neurotypical brains.

Methods: Forty-four patients with SLD and 44 healthy controls aged 7–12 years were included. SLD diagnosis and severity was determined using DSM-5–based interviews and SLD clinical observation battery. Serum neurotrophins were measured using enzyme–linked immunosorbent assay.

Results: BDNF (p=0.032), NGF (p=0.029), and NT-3 (p=0.025) serum levels were significantly higher in the SLD group compared to the control group; however, serum levels of GDNF did not show any significant difference between groups. On the other hand, GDNF serum levels were significantly different between mild and severe SLD groups (p=0.007) and were lower in severe SLD subjects than in mild cases. There was also a significant correlation between patients' reading speeds and serum levels of GDNF (p=0.025), and GDNF serum levels were lower in patients with slower reading speeds.

Conclusion: These findings suggest that neurotrophins might play a role in the pathophysiology of SLD. Increased serum levels of BDNF, NGF, and NT-3 might reflect compensatory attempts at neuroprotection against neurodevelopmental impairment.

Keywords: Keywords: Brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor, neurotrophin 3, Specific learning disorder

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INTRODUCTION

Specific learning disorder (SLD) is a neurodevelopmental disorder defined by difficulties in learning and with academic skills including reading, spelling, writing, calculating, and mathematical reasoning for at least six months despite appropriate intervention. The prevalence of SLD across these academic domains worldwide is 5–15% among school-age children (1). The etiology of SLD involves complex interactions of genetic, epigenetic, and environmental factors (2). Postmortem examinations and animal studies suggest that the impairment of neuronal migration has a role in the neurobiological origin of SLD (3,4). Despite increasing evidence that suggests the roles of neuroanatomic and neurobiological systems in the etiology of SLD, the underlying precise mechanisms remain elusive. Identifying the biomarkers involved in SLD may likely contribute to understanding the etiopathogenesis of this disorder.

Neurotrophic factors are polypeptide growth factors that regulate neurogenesis, neuronal survival, neuroplasticity, and neuronal migration (5,6). Neurotrophins can cross the blood–brain barrier with transporters, and their serum levels are thought to correlate with their levels in the brain (7). To date, the roles of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and glial-derived neurotrophic factor (GDNF) have been investigated with regard to various neurodevelopmental disorders. BDNF is the most abundant neurotrophic factor in the central nervous system, and therefore it has been the most studied neurotrophin in neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), intellectual disability (ID), and language disorder (LD) (8–15). Several studies have demonstrated increased peripheral BDNF levels in patients with ADHD, ASD and ID; however, other studies have reported no altered levels of BDNF in ADHD patients and have shown lower levels of BDNF in patients with ASD compared to control subjects (8–13). Similarly, lower peripheral BDNF levels were reported in children with LD (15). NGF plays a crucial role in the development and functional integrity of cholinergic neurons that take part in learning, memory, and attention (6). The possible role of NGF is implicated in the pathophysiology of neurodevelopmental

Correspondence Address: Gökçe Sultan Uncu, Van Eğitim ve Araştırma Hastanesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları, Van, Turkey • **E-mail:** gokce.uncu@istanbul.edu.tr
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Highlights

- **Definite mechanisms of specific learning disorder (SLD) remain mostly unknown.**
- **BDNF, NGF, and NT-3 might be neuroprotective against neurodevelopmental impairment.**
- **GDNF might be associated with the severity of SLD.**
- **Neurotrophins might play a role in the pathophysiology of SLD.**

disorders such as ADHD, LD, and global developmental delay (GDD) (15–17). Increased NGF serum levels in children with ADHD were reported; however, subsequent studies found no alteration of NGF serum levels in ADHD patients (9,10,16).

NT-3, a neurotrophin, is widely expressed in developing brains, especially in newborns' hippocampus and immature cortical regions (18). The role of NT-3 was also investigated in relation to several neurodevelopmental disorders, such as ADHD, ASD, and LD (9–11,15,19,20). Bilgic et al. reported higher NT-3 serum levels in children with ADHD, but another study failed to show any relation (9,10). A number of researchers have reported lower NT-3 serum levels in ASD patients, but another attempt has shown no association of NT-3 with ASD (11,19,20). Bilgic et al. also reported lower NT-3 serum levels in LD patients (15). GDNF is found widespread in the brain and has an important role in protecting the dopaminergic and serotonergic pathways against oxidative and inflammatory damage via its neuroprotective effects (21). Several studies that investigated the role of GDNF in ADHD documented higher circulatory levels of GDNF compared to healthy controls (9,10). Although further work is required to confirm the findings, recent evidence suggests that GDNF may have a role in neurodevelopmental disorders.

Despite extensive research on the roles of BDNF, NGF, NT-3, and GDNF in several neurodevelopmental disorders, according to our current knowledge, only BDNF levels were investigated in children with SLD. A study that investigates the relationship between SLD and serum BDNF levels reported no difference in serum BDNF levels between children with SLD and the healthy controls (22). On the other hand, various animal studies suggested that BDNF, NGF, NT-3, and GDNF play important roles in cognitive functions such as learning and memory (23–26). Although the role of neurotrophins has been implicated in learning and other cognitive functions during animal studies, a direct correlation between them and SLD could not be established. The fundamental aim of this study was to determine whether serum levels of BDNF, NGF, NT-3, and GDNF in children with SLD differ from neurotypical control subjects and to evaluate the relationship between neurotrophic factor levels and clinical features of SLD. It is hypothesized that neurotrophic factor levels may be different in patients with SLD, based on the knowledge that these factors are involved in neurogenesis, neuroplasticity, and neuronal migration; and faults in these processes are potentially implicated in the etiology of SLD. Therefore, the levels of these neurotrophic factors may be potential neurobiological markers for SLD.

METHODS

Participants

Subjects were recruited from the outpatient clinic of the Department of Child and Adolescent Psychiatry at the Istanbul Faculty of Medicine. Children with a diagnosis of SLD according to DSM-5 criteria were included in this study. Exclusion criteria included the presence of major neurological/physical illness (epilepsy, metabolic/genetic diseases, visual or hearing disability, etc.), ADHD, ASD, or an intelligence quotient (IQ) score below 80 according to the Turkish version of the Wechsler Intelligence Scale for Children-Revised (WISC-R) (27). Children who had used any psychotropic drug within the previous year were also excluded. The control group was chosen from outpatient clinics of the pediatrics at the Istanbul Faculty of Medicine among healthy children who were referred for routine medical checkups. This study was approved by the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (Case Number: 2019/585, Date: 26/04/2019) and all procedures were in accordance with the standards in the Declaration of Helsinki.

Instruments

Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version–DSM-5 (K-SADS-PL-DSM-5): The purpose of this semi-structured interview is to determine the present and lifetime psychopathology in children and adolescents. The updated version of K-SADS-PL according to DSM-5 criteria was adapted to the Turkish population by Ünal et al (28).

Specific Learning Disorder (SLD) Clinical Observation Battery: This concept was developed by Korkmazlar in 1992 and revised to include new subscales (29). This battery includes nine subscales that evaluate arithmetic skills, reading skills, Gessell figures, writing skills, ability to draw a clock, right/left discrimination, lateralization, before/after relationships, and ordering. Each subscale is defined as being consistent with SLD or being inconsistent with SLD (29,30). In this study, we also evaluated the severity of impairment in reading according to the data obtained from the SLD observation battery. We specified the severity of impairment in reading according to the reading speeds of the children. We obtained numeric data by dividing the reading speed (number of words read in one minute) of the SLD cases to mean standard values of reading speed according to grade, which was defined in the SLD clinical observation battery (31).

Wechsler Intelligence Scale for Children-Revised (WISC-R): This intelligence scale was developed to evaluate IQ levels of children between 6 and 16 years. It was adapted to the Turkish population by Savaşır and Şahin (27). This scale consists of verbal and performance parts. IQ scores are defined in three parts: verbal IQ, performance IQ, and full–scale IQ.

Conners' Parent Rating Scale–Revised Short (CPRS-RS): This 27-item, four-point Likert-type scale evaluates ADHD symptoms as well as other behavioral problems in children and adolescents. CPRS-RS includes inattention/cognitive problems, hyperactivity, and oppositional behavior subscales and allows the screening of cognitive problems and the exclusion of ADHD. Both the reliability and the validity of CPRS-RS have been confirmed for the Turkish population (32).

Diagnostic and Symptom Assessment

Children who had been diagnosed with SLD according to DSM-5 criteria and did not meet any exclusion criteria were enrolled in this study after the verbal assent of children and written informed consent of the parents were both provided following a complete description of the study. In addition to DSM-based clinical interviews, SLD diagnoses were supported by an SLD clinical observation battery. To exclude any intellectual disabilities, WISC-R was performed with all participants. These tests were conducted by psychologists who had specific training and experience in the application of these tests. All participants were screened for psychiatric disorders by child and adolescent psychiatrists, using the K-SADS-PL-DSM-5. Parents completed the CPRS-RS and a sociodemographic data form developed by the researchers. The control subjects were also included after the consent of parents and children. All

of the psychiatric and psychometric assessments that applied to the case group were also carried out on the control group to determine exclusion criteria and to identify possible comorbid disorders.

Blood Sampling

Venous blood samples were obtained between 8:00 and 10:00 a.m. to avoid circadian alteration. Participants were also notified to avoid heavy exercise, eating, and drinking prior to sampling. Samples of venous blood were stored in biochemistry tubes. The biochemistry tubes were consistently centrifuged at 3000–4000 rpm for 15 min, and the aliquots of serum specimens were kept at -80 °C until the day of analysis. Serum levels of BDNF, NGF, NT-3, and GDNF were measured by the enzyme–linked immunosorbent assay (ELISA) method using commercial human ELISA test kits and following the protocols of manufacturers (Boster Biological Technology, CA, USA; Bioassay Technology Laboratory, Shanghai, China; USCN, Hubei, China; Bioassay Technology Laboratory, Shanghai, China, respectively).

Statistical Analysis

Statistical analyses were conducted using the IBM SPSS Statistics 21.0 package program (IBM Corp. Released 2012, Armonk, NY, USA). Categorical data were presented as counts and frequencies; non-normally distributed continuous data were presented as medians and 25–75 percentiles, and normally distributed continuous data were presented as means and standard deviations. Comparisons of categorical variables between groups were performed using a chi-square test and Fisher's exact test when needed. The Shapiro–Wilk test was used to check whether continuous variables in patients and controls were normally distributed. Continuous variables that were non-normally distributed were analyzed using the Mann–Whitney U test, and in other cases where the data were normally distributed, the Student's t-test was conducted. The correlation of continuous variables was analyzed by Spearman's rank correlation analysis. The Kruskal–Wallis test was performed to compare more than two independent groups for non-normally distributed continuous data. When any statistically significant difference was detected, the Dunn–Bonferroni post hoc pairwise test was performed to determine which pairs of groups differed from each other. Probability values (p) smaller than 0.05 were regarded as statistically significant.

RESULTS

A total of 59 children with SLD were approached. In the case group, two children declined to give blood samples and, 13 children were excluded according to the exclusion criteria. Of the control group comprising 53 children, one declined to give a blood sample and eight were excluded based on the exclusion criteria. The final case group comprised of 44 children with SLD, and the final control group comprised of 44 neurotypical controls, matched to the case group according to age and gender. There was no significant difference between groups regarding age, sex, and body mass index (BMI) distributions. The sociodemographic characteristics of children with SLD and the control group are reported in Table 1.

Testing revealed that 56.8% (n=25) of children with SLD diagnoses had specific impairments in reading, written expression, and mathematics; 29.5% (n=13) had specific impairments in reading and written expression; 9.1% (n=4) had specific impairment only in reading; 2.3% (n=1) of them had specific impairments in reading and mathematics, and 2.3% (n=1) had specific impairments in written expression and mathematics. This sample contained no children diagnosed with impairment in only

Table 1. The sociodemographic characteristics of children with specific learning disorder (SLD) and the control group

Variables		Case group (n=44)	Control group (n=44)	z/χ^2	p
Age (years)		9.46 (7.96–10.17)	8.70 (7.75–9.92)	-0.981[a]	0.327[a]
Gender					
	Male	34 (77.3%)	34 (77.3%)		
	Female	10 (22.7%)	10 (22.7%)		
Educational level of mother				33.808[b]	<0.001[b]
	Illiterate	7 (15.9%)	0 (0.0%)		
	Primary school graduate	16 (36.4%)	3 (6.8%)		
	Secondary school graduate	5 (11.4%)	1 (2.3%)		
	High school graduate	13 (29.5%)	18 (40.9%)		
	University or higher graduate	3 (6.8%)	22 (50.0%)		
Educational level of father				30.192[b]	<0.001[b]
	Illiterate	3 (6.8%)	0 (0.0%)		
	Primary school graduate	21 (47.7%)	7 (15.9%)		
	Secondary school graduate	11 (25.0%)	3 (6.8%)		
	High school graduate	5 (11.4%)	11 (25.0%)		
	University or higher graduate	4 (9.1%)	23 (52.3%)		
Family Income Status				16.565[b]	<0.001[b]
	Below minimum wage	18 (%40.9)	2 (%4.5)		
	Above minimum wage	26 (%59.1)	42 (%95.5)		
Birth Weight				0.917[b]	0.338[b]
	<2500 gr (low birth weight)	30 (68.2%)	34 (77.3%)		
	2500 gr or more	14 (31.8%)	10 (22.7%)		
Gestational age					0.616 [c]
	≤37th week (premature)	43 (97.7%)	41 (93.2%)		
	37th week or more	1 (2.3%)	3 (6.8%)		

Continuous data are presented as median and as 25–75 percentiles.
a: Mann-Whitney U test; b: Chi-Square test; c: Fisher's exact test.

Table 2. Clinical characteristics of the children with specific learning disorder (SLD) and the control group

Variables		Case Group (n=44)	Control Group (n=44)	t/z	p
WISC-R					
	PIQ	103.35±13.870	111.39±13.678	2.627[a]	0.010
	VIQ	89.70±11.665	106.93±10.086	7.116[a]	<0.001
	PIQ – SIQ discrepancy	13.65±14.852	4.46±13.278	-2.936[a]	0.004
	FSIQ	95.00 (88.00–103.00)	108.00 (102.50–115.75)	-5.131[b]	<0.001
CPRS-RS					
	IA/CP	5.00 (4.00–8.00)	2.00 (0.25–3.00)	-4.969[b]	<0.001
	HA	1.00 (0.00–2.00)	1.00 (0.00–4.00)	-0.792[b]	0.428
	OB	2.00 (1.00–4.00)	2.50 (0.00–5.75)	-0.502[b]	0.616

CPRS-RS: Conners' Parent Rating Scale-Revised Short; FSIQ: full scale intelligence quotient; HA: hyperactivity; IA/CP: inattention/cognitive problems; OB: oppositional behavior; PIQ: performance intelligence quotient; VIQ: verbal intelligence quotient; WISC-R: Wechsler Intelligence Scale for Children-Revised.
Continuous data are presented as median and 25–75 percentiles in Mann-Whitney U test, as mean ± standard deviation in Student t test.
a: Student t test. b: Mann-Whitney U test.

Table 3. Serum levels of neurotrophic factors for the children with specific learning disorder (SLD) and the control group

Variables	Case group (n=44)	Control group (n=44)	z	p
BDNF (ng/ml)	2.760 (1.573–3.160)	2.170 (1.045–2.960)	-2.149[a]	0.032
NGF (pg/ml)	296.505 (200.100–331.212)	229.705 (170.070–305.750)	-2.187[a]	0.029
NT-3 (pg/ml)	38.910 (33.440–52.600)	35.795 (32.180–39.353)	-2.237[a]	0.025
GDNF (ng/ml)	1.520 (1.263–2.875)	1.821 (1.445–7.071)	-1.532[a]	0.126

BDNF: Brain-Derived Neurotrophic Factor; GDNF: Glial-Derived Neurotrophic Factor; NGF: Nerve Growth Factor; NT-3: Neurotrophin 3.
Continuous data are presented as median and 25–75 percentiles.
a: Mann-Whitney U Test.

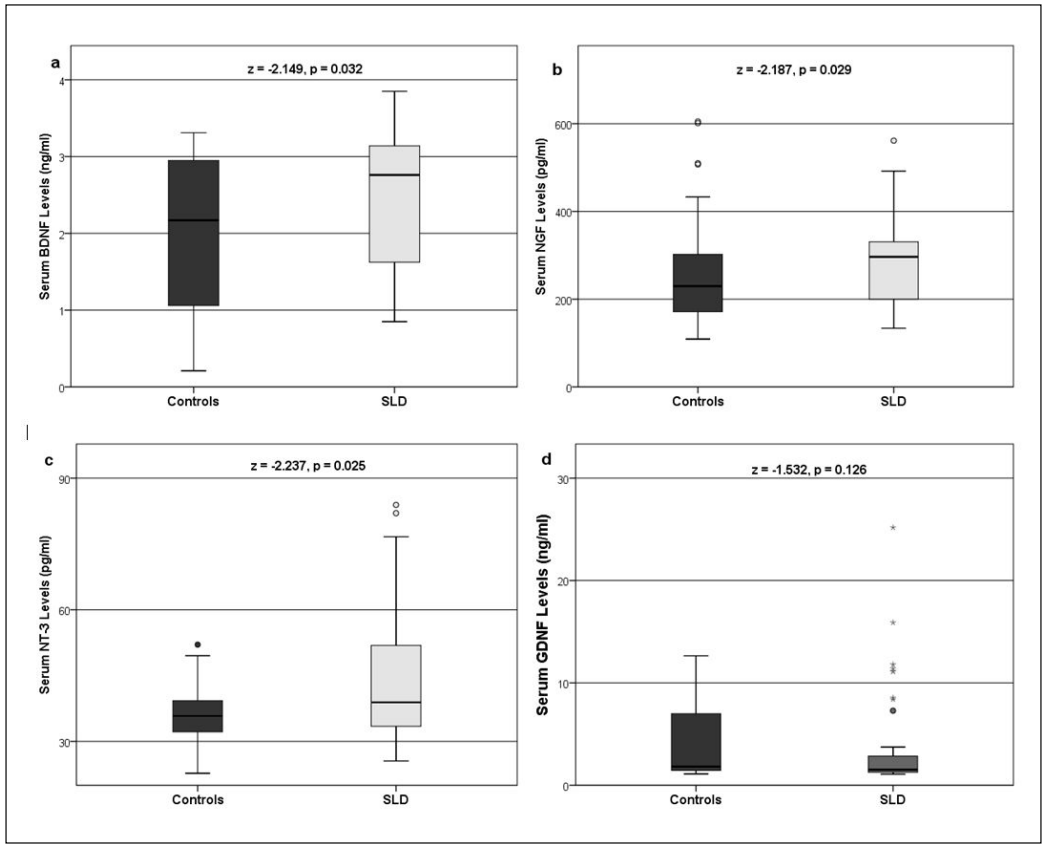

Figure 1. a–d. Box plots representing the distribution of serum BDNF **(a),** NGF **(b),** NT-3 **(c),** and GDNF **(d)** levels in children with SLD and healthy controls (BDNF: brain-derived neurotrophic factor; GDNF: glial-derived neurotrophic factor; NGF: nerve growth factor; NT-3: neurotrophin 3. Mann-Whitney U test was used for comparisons between two groups).

Table 4. Comparison of neurotrophic factor serum levels between subgroups of specific learning disorder (SLD)

Variables	Mild SLD (n=20)	Moderate SLD (n=11)	Severe SLD (n=13)	p
BDNF (ng/ml)	2.615 (1.558–3.080)	2.850 (1.300–3.850)	2.810 (2.185–2.970)	0.790[a]
NGF (pg/ml)	301.520 (202.983–335.050)	232.130 (200.00–314.360)	318.990 (232.610–355.700)	0.266[a]
NT-3 (pg/ml)	37.665 (33.435–50.470)	44.790 (33.120–54.570)	39.430 (33.910–54.890)	0.931[a]
GDNF (ng/ml)	2.550 (1.455–8.513)	1.520 (1.330–2.665)	1.290 (1.195–1.485)	0.009[a]

BDNF: Brain-Derived Neurotrophic Factor; GDNF: Glial-Derived Neurotrophic Factor; NGF: Nerve Growth Factor; NT-3: Neurotrophin 3.
Continuous data are presented as median and 25–75 percentiles.
a: Kruskal-Wallis Test.

written expression or only mathematics. Verbal (VIQ), performance (PIQ), and full–scale IQ (FSIQ) scores were lower in the SLD group than in the control group (t=7.116, p=<0.001; t=2.627, p=0.010; z=-5.131, p=<0.001, respectively). The verbal-performance IQ discrepancy was higher in the SLD group in comparison to the control group (z=-2.936, p=0.004). The scores for inattention/cognitive problems found using CPRS-RS were higher in the SLD group in comparison to the control group (z=-4.969, p=<0.001). Hyperactivity and oppositional behavior subscale scores were not statistically different between groups. According to K-SADS-PL, the most frequent comorbid psychiatric disorder in the SLD group was specific phobia (n=10), followed by social anxiety disorder (n=5), enuresis (n=5), separation anxiety disorder (n=2), generalized anxiety disorder (n=1), and major depressive disorder (n=1) at present. In the control group, the most frequently diagnosed psychiatric disorder was specific phobia (n=9), followed by generalized anxiety disorder (n=2), tic disorders (n=2), enuresis (n=1), and obsessive compulsive disorder (n=1) at present. Across a lifetime, social anxiety disorder (χ^2=5.091, p=0.024) and enuresis (χ^2=7.221, p=0.007) were more common in the SLD group compared to the control group. The clinical characteristics of the children with SLD and the control group are reported in Table 2.

The relationships among neurotrophin serum levels and age, sex, and BMI were assessed in children with SLD. None of the neurotrophic factor levels were correlated with the age, sex, or BMI levels of the children in the SLD group. When groups were compared for neurotrophic factor serum levels, BDNF (z=-2.149, p=0.032), NGF (z=-2.187, p=0.029), and NT-3 levels (z=-2.237, p=0.025) were significantly higher in children with SLD. Serum levels of GDNF did not differ between groups. Serum levels of neurotrophic factors for children with SLD and the control group are reported in Table 3 and the box plots representing their distribution are reported in Figure 1.

We specified the current severity of SLD as mild, moderate, or severe according to DSM-5 criteria; 45.5% (n=20) had mild SLD, 25% (n=11) had moderate SLD, and 29.5% (n=13) had severe SLD. When SLD subtypes were compared with regard to neurotrophic factor levels, there were no significant differences between BDNF, NGF, and NT-3 levels among these subgroups; however, GDNF levels were significantly different between specified subgroups (p=0.009) (see Table 4). Post–hoc analyses showed a significant difference in GDNF levels between mild and severe groups (p=0.007). Serum levels of GDNF were higher in children with mild SLD compared to the severe group. We also evaluated the severity of impairment in reading according to the reading speeds of children. In a similar way, a positive correlation was detected between GDNF levels and the reading speeds of children (r=0.338, p=0.025).

Furthermore, the correlations among neurotrophin serum levels and IQ scores, as well as CPRS-RS scores were assessed in children with SLD. There were no correlations between IQ scores and BDNF, NGF, nor NT-3 levels. However, GDNF was positively correlated with full-scale IQ scores (r=0.396, p=0.008). There were no correlations between CPRS-RS subscale scores and BDNF/NGF levels, whereas GDNF serum levels were negatively correlated with inattention/cognitive problems scores (r=-0.315, p=0.037) and NT-3 levels were positively correlated with oppositional behavior scores (r=0.339, p=0.025).

DISCUSSION

This case-control study investigated the relationship between neurotrophic factors and SLD. Further analyses showed statistically significant higher serum levels of BDNF, NGF, and NT-3 in children with SLD. Additionally, in terms of the relationship between SLD severity and serum neurotrophic factor levels, serum GDNF levels were higher in children with mild SLD compared to the group with severe SLD. Also, serum levels of GDNF were higher in SLD subjects whose reading speeds were faster.

Regarding sociodemographic characteristics, the family income and educational level of both parents were lower in the SLD group in comparison to the control group. A significant relationship between SLD and lower educational levels of mothers, as well as between SLD and lower family income was reported in large-scale national research (33). Lower educational levels in parents could be related to possible diagnoses of SLD in parents and the disruption of education due to difficulties regarding SLD. Since income and educational levels are related, we could also expect to observe lower family income in families of children with SLD.

Findings showed higher serum levels of BDNF in children with SLD than in neurotypical control subjects. Although some animal studies have suggested that decreased BDNF in the brain is associated with impairments in learning and memory functions, a recent study showed no relationship between SLD and serum BDNF levels (22,23). Structural and functional brain changes, such as cortical dysplasias, neuronal ectopias, and symmetry or reverse asymmetry in normally asymmetrical planum temporale, were implicated in the etiology of dyslexia. According to our current understanding, these cortical anomalies result from impairments in neuronal migration during the embryonic period (3,34). Additionally, in animal studies, it has been shown that candidate dyslexia susceptibility genes such as KIAA0319, DYX1C1, and DCDC2 play a role in the regulation of neuronal migration during the development of the neocortex (4,35,36). BDNF also plays a critical role in neurogenesis and neuronal migration (37). Considering that defects in neuronal migration cause structural abnormalities in the dyslexic brain, one could expect to observe lower BDNF levels in patients with SLD. However, higher BDNF levels in SLD patients could be related to a compensatory mechanism for neuroprotection. Similarly, numerous studies have stated increased circulating BDNF levels in neurodevelopmental disorders such as ADHD and ASD (8,11,12). In addition to the research that found increased peripheral BDNF levels in ASD, a neurodevelopmental disorder, Kasarpalkar et al. reported higher serum BDNF levels in atypical autistic subjects with

clinically milder phenotypes in comparison to control subjects but not in typical ASD subjects with clinically severe phenotypes (11,12,14). The authors reported lower BDNF serum levels in females with Rett syndrome/typical autism. Similarly, the authors inferred that higher BDNF levels may imply a protective response, and lower levels may lead to impairment in the neuroprotective mechanism (14).

To our knowledge, as the first study to examine the circulating levels of NGF in children with SLD, our results demonstrated significantly increased NGF serum levels in children with SLD. Animal studies that investigated the role of NGF in learning and memory functions suggested that decreased NGF levels cause impairment in learning and memory (23,24). In view of the role of NGF in learning, it may be proposed that the higher serum levels of NGF observed in our study could indicate increased NGF levels in the brain as a compensatory attempt to protect against neurodevelopmental impairment. Similar to our results, Güney et al. reported increased NGF serum levels in patients with ADHD, a neurodevelopmental disorder (16). The study's authors suggested that NGF levels might be increased due to a compensatory and neuroprotective mechanism against structural and functional changes in the ADHD brain (16). As it is well known that there are structural and functional abnormalities in the dyslexic brain, higher serum levels of NGF in SLD subjects demonstrated in our study might have been developed as a result of a similar mechanism (3,34,38). The role of NGF was also investigated in other neurodevelopmental disorders such as GDD. In a recent study that investigated the effects of intrauterine antiepileptic exposure on development and its relationship with NGF, an increased risk of GDD and lower global development scores were observed when exposed to antiepileptics in the intrauterine period, and there was a positive correlation between global development scores and NGF serum levels (17). Although it is still uncertain whether NGF has any role in the emergence of neurodevelopmental disorders, decreased levels of NGF were demonstrated in this research that has examined the role of NGF in developmental delay (17). This variation might be due to the different etiological origins of each neurodevelopmental disorder, even if they are under the same category according to nomenclature.

Our study showed significantly higher NT-3 serum levels in children with SLD than in the control group. To date, no other study has investigated circulating NT-3 levels in SLD patients according to our knowledge. However, some animal studies have been carried out on the role of NT-3 in learning and memory. Evidence from those studies demonstrated that lacking NT-3 genes caused deficits in learning and memory functions in rats and it was suggested that NT-3 may decrease impairment in learning and memory functions via its neuroprotective effect (25,39). On the basis of this evidence, it may be suggested that NT-3 levels were increased to exert neuroprotective effects against deterioration in the neurodevelopmental period of SLD patients. Similarly, higher NT-3 serum levels were reported in patients with ADHD, a neurodevelopmental disorder (9). Bilgiç et al. proposed that exposure to stress such as perinatal and psychological stress, may provoke the production and/or secretion of NT-3, which has an important role in maintaining homeostasis during stressful conditions. Such stressful conditions may be the pathophysiological link between ADHD, which has well-known associations with perinatal and psychological stress, and elevated NT-3 levels (9). As the role of perinatal risk factors; such as preterm birth, prenatal nicotine exposure, and perinatal asphyxia has been also indicated in the etiopathogenesis of SLD (40–43), it is possible that higher NT-3 levels in SLD patients could have been a consequence of a similar mechanism. However, considering that the relationship between perinatal factors, NT-3 levels, and SLD had not been investigated in our research, it is not possible to confirm that this mechanism is the cause of our results within the scope of our study.

This study found no significant difference between SLD patients and control subjects with regard to serum levels of GDNF. However, we detected a statistically significant relationship between SLD severity and GDNF levels. As a result, we observed that GDNF serum levels were lower in patients with severe SLD than in patients with mild SLD. Similarly, we found a significant relationship between the severity of impairment in reading and GDNF levels; serum levels of GDNF were reduced in patients with slower reading speeds. To date, GDNF levels have not been investigated pertaining to SLD, according to the authors' knowledge. Lower GDNF levels in severe cases may indicate a defect in the neuroprotective mechanism, while higher levels in mild SLD cases may suggest a protective response. The role of GDNF was researched in other neurodevelopmental disorders such as ADHD. Several case-control studies also demonstrated higher GDNF serum levels in ADHD patients than in the control subjects (9,10). Thus far, it remains uncertain whether the mechanism for increased GDNF levels serves as a pathological and/or compensatory attempt (9).

We observed a statistically significant positive correlation between GDNF serum levels and full–scale IQ scores in SLD patients. Observing increased levels of GDNF in patients with higher IQs strengthens the hypothesis that GDNF has a neuroprotective role in development. Similar findings were reported in a study that examined the relationship between GDNF and developmental problems (44). İbili Ucuz et al. investigated the Denver Developmental Screening Test-II (DDST-II) scores and serum GDNF levels of children with GDD at the time of diagnosis and six months after the start of educational intervention. As a result, the children demonstrated an increase both in DDST-II scores and in GDNF levels after educational intervention, which suggests that GDNF might play a role in GDD (44). Our findings regarding the association between GDNF and IQ levels seem compatible with the current literature.

According to the current knowledge of the authors, this is one of the first studies which investigates the relationship between neurotrophic factors and SLD. The strongest point of this research is filling a gap in the literature by examining this relationship. Excluding the cases and controls with neurologic/metabolic/genetic disease, ADHD, and ASD ruled out major confounding factors and allowed the researchers to obtain specific results for SLD. However, this study also had several limitations, including a relatively small sample size. Another limitation is that we could not exclude the subjects with internalizing disorders, such as anxiety disorders, due to having a smaller sample size resulting from the numerous exclusion criteria. However, most of the children with any psychiatric diagnosis had only specific phobias, and the percentage of psychiatric diagnoses was both relatively low and mild in severity. In addition, this study was designed as a cross-sectional study, therefore it is not possible to determine a causal relationship between neurotrophic factor levels and SLD. Furthermore, even though we used a range of psychometric tests such as WISC-R and SLD clinical observation battery, the fact that we were not able to use the most recent versions of these psychometric tests could be considered a limitation. Finally, we still do not know the most reliable method to measure neurotrophic factors, so we measured the serum levels of neurotrophins from participants' blood samples using the ELISA method.

In conclusion, our findings suggest that neurotrophic factors might play a role in the pathophysiology of SLD. The elevation of serum levels of BDNF, NGF, and NT-3 might be compensatory attempts at neuroprotection against neurodevelopmental impairment. To confirm our findings, repetitions in a larger sample (considering the limitations of the present study) are required. Further studies that combine neuroimaging and genetic assessments of SLD subjects could be useful to enlighten the etiopathogenesis of SLD.

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Ethics Committee Approval: This study was approved by the İstanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (Case Number: 2019/585, Date: 26/04/2019) and all procedures were in accordance with the standards in the Declaration of Helsinki.

Informed Consent: Verbal assent of children and written informed consent of the parents were both provided following a complete description of the study.

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