

Inflammatory and Biochemical Concepts in Children with Specific Learning Disorders: A Comparative Study

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ABSTRACT

This study aimed to investigate the role of inflammation and biochemical parameters in children with Specific Learning Disorders (SLD) and to evaluate these parameters based on the severity of SLD. The study was planned as a retrospective. 39 children diagnosed with SLD and 32 healthy controls aged 6-16 years, who had hemogram and biochemistry tests performed at their admission, were included in the study. Diagnoses were based on DSM-5 criteria, clinical interviews, family interviews, and assessments of reading, writing, and math skills. The study received ethical approval from the Cumhuriyet University Non-Invasive Clinical Research Ethics Committee. Lymphocyte count and WBC were significantly higher in the SLD group ($p = 0.003$, $p = 0.006$, respectively). A significant difference was detected between the groups regarding platelet/lymphocyte ratio (PLR) ($p = 0.047$). No significant differences were found in blood parameters (erythrocyte, neutrophil, lymphocyte, WBC, platelet, T4, TSH, folic acid, vitamin B12, PLR, neutrophil/lymphocyte ratio (NLR)) when evaluated according to the severity of SLD. The results of our study suggest that inflammation may play a role in SLD, but further research with larger sample sizes, longitudinal designs, and comprehensive assessments of inflammatory markers is needed to better understand these associations.

Keywords: SLD, PLR, NLR, Inflammation, Biochemical markers.

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Introduction

Neurodevelopmental disorders constitute a heterogeneous group of inherited medical conditions that primarily impact social communication, language, attention, impulsivity, learning, perception, and motor coordination. The condition has a detrimental impact on both the individual and the family, persisting from childhood to adulthood [1]. There is mounting evidence that inflammatory processes are involved in the etiology of neurodevelopmental disorders, including autism, specific learning disorders, attention deficit hyperactivity disorder, emotional disorders such as depression and bipolar disorder, and tic disorders. Neuroinflammation has been linked to alterations in brain development, including modifications to synaptic plasticity and synaptogenesis [2]. Specific learning disorder (SLD) is a neurodevelopmental disorder that is characterized by a failure to achieve the expected level of proficiency in reading, mathematics, and written expression skills when considering the individual's chronological age, educational level, and intelligence. Despite its prevalence in childhood, the etiology of SLD remains unclear [3]. Proposed mechanisms include neuronal damage and degeneration, increased oxidative stress, decreased neurotrophic support, glial activation, changes in neurotransmitter metabolism, and disruption of the blood-brain barrier [4]. Consequently, the potential contribution of inflammation to neurodevelopmental disorders is being subjected to further scrutiny. The

hematological system is a vital organ for human immune defense and plays a central role in the inflammatory process and sepsis [5]. Leukocytes, endothelium, platelets (PLTs), and numerous other components are responsible for the activation of the immune system. An increase in the inflammatory response triggered by environmental factors in the early stages of development may result in structural and/or functional alterations in brain development, which may subsequently lead to the onset of neurodevelopmental disorders such as specific learning disabilities (SLD) and attention deficit hyperactivity disorder (ADHD) [6]. The neutrophil/lymphocyte ratio (NLR), mean platelet volume (MPV), monocyte/lymphocyte ratio (MLR), and red blood cell distribution width (RDW) are frequently employed as straightforward peripheral inflammation markers that can reflect the underlying state of systemic inflammation and can be readily and rapidly measured in whole blood [10]. The utilization of these cells as markers has facilitated the identification of alterations in inflammatory status across a spectrum of psychiatric disorders [11-16]. These psychiatric disorders include schizophrenia, bipolar disorder, autism spectrum disorders, and attention deficit hyperactivity disorder [4, 7-9]. Furthermore, vitamins, minerals, and hormones are implicated in the etiology, disease process, and treatment of psychiatric disorders [10]. It is assumed that B12, folic acid and thyroid hormones exert a direct effect on cognitive functions,

especially on the energy metabolism of neurons and glial cells, the synthesis of neurotransmitters and receptor binding. [11].

A review of the literature reveals a paucity of studies examining the relationship between SLD and inflammation. Nevertheless, no studies have examined the role of SLD and inflammation and biochemical parameters together, nor have they evaluated these factors according to SLD severity. In light of these findings, the objective of our study was to examine the role of SLD and inflammation and biochemical parameters in patients with specific learning disorders, evaluating them according to SLD severity.

Materials and Methods

The records of the child and adolescent mental health and diseases outpatient clinic between May 2023 and May 2024 were retrospectively examined and a diagnosis of SLD was made as a result of a clinical interview based on DSM-5, family interview, psychiatric examination including assessment of reading, writing and mathematics skills appropriate for the age and grade level, and teacher information form evaluation. Thirty-nine children aged between 6 and 16 years with no accompanying psychiatric, neurological and medical diseases, no mental retardation and no mental retardation, who underwent haemogram and biochemistry examinations, and 32 age- and gender-matched healthy children who were not diagnosed at their first presentation to child and adolescent mental health and diseases and who underwent haemogram and biochemistry examinations were included as the control group. Routine blood tests were requested from patients at the first application in order to organize diagnosis and treatment decisions and to make a differential diagnosis. Another purpose was to see the current metabolic status and to observe the side effects of drugs if any medication was to be started. The severity of SLD was determined as a result of a clinical interview based on DSM-5. Sociodemographic

characteristics registered in the system were used. The minimum sample size was determined by power analysis. The study was started after approval was obtained from the Cumhuriyet University Non-interventional Clinical Research Ethics Committee (dated 16.05.2024, numbered 2024/05-14). When $\beta=0.10$ and $\alpha=0.05$ $1-\beta=0.90$ was taken in the study, it was decided to include 39 individuals in the patient group and 32 individuals in the control group. The power of the test was found as $p=0.90498$. Hormone and vitamin results; Roche Cobas 8000 system (Roche Diagnostics, Mannheim, Germany). Complete Blood Count (CBC); CBC MINDRAY 5200 (China) was analyzed in the hospital biochemistry laboratory.

Statistical Methods

The data obtained from our study were evaluated with SPSS 23.0 program. The normality of the data was checked with Kolmogorov-Smirnov test. Since the data provided parametric conditions, it was analyzed with independent sample t test for two independent groups and F test (ANOVA) for more than two groups. When ANOVA was used in more than two group comparisons, Tukey's T2 test was used for those that provided the homogeneity assumption and Tamhane's T2 test was used for those that did not provide the homogeneity assumption to determine which group was different from the others. Chi-square test was used for categorical variables. The error level will be taken as 0.05.

Results

Demographic Characteristics

This study was conducted with 39 children with SLD and 32 healthy control groups. Table 1 shows the socio-demographic and clinical characteristics of the SLD groups and controls. There was no significant difference between the groups in terms of age, age groups, gender, place of residence, family income level, and mother and father's education level (all p values >0.05) (Table 1).

Table 1. Sociodemographic characteristics of the sample

	SLD group (N=39)	Control group (N=32)	p-value*
Age (mean-years±SD)	10.41±2.67	11.03±2.86	0.348
Gender (n,%)			0.934
Male	24 (61.5)	20 (62.5)	
Female	15 (38.5)	12 (37.5)	
Place of residence (n,%)			0.994
Urban	28 (71.8)	23 (71.9)	
Rural	11 (28.2)	9 (28.1)	
Family income level (n,%) [†]			0.712
The minimum wage/less than minimum wage	20 (51.3)	15 (46.9)	
Above the minimum wage	19 (48.7)	17 (53.1)	
Education level of mother (n,%)			0.292
Primary education and lower	30 (76.9)	21 (65.6)	
Upper primary education	9 (23.1)	11 (34.4)	
Education level of father (n,%)			0.621
Primary education and lower	23 (59.0)	17 (53.1)	
Upper primary education	16 (41.0)	15 (46.9)	

*The chi-square test for categorical variables and the Independent-samples t-test for continuous variables were used to test group differences.

Bold font indicates statistical significance: $p < 0.05$

[†]The level of income was determined by the minimum wage value on the date of the study.

Abbreviations: SD, Standard Deviation; SLD, Specific Learning Disorder.

Comparison of Hemogram and Biochemical Parameters Between Two Groups

Table 2. Comparison of hemogram and biochemical parameters between two groups.

	SLD group (N=39)	Control group (N=32)	p-value*
Erythrocyte (10 ³ /uL) (mean±SD)	4,98±0,48	5,00±0,41	0,957
Neutrophil (10 ³ /uL) (mean±SD)	4,17±1,68	3,64±1,12	0,127
Lymphocyte (10 ³ /uL) (mean±SD)	2,96±0,95	2,32±0,72	<i>0,003</i>
WBC (10 ³ /uL) (mean±SD)	7,91±2,37	6,54±1,41	<i>0,006</i>
Platelet(10 ³ /uL) (mean±SD)	337,21±67,86	308,72±66,33	0,080
T4 (mcg/dl) (mean±SD)	1,28±0,16	1,29±0,35	0,879
TSH (mIU/L) (mean±SD)	2,31±2,42	2,42±1,58	0,729
Folic acid(ng/ml) (mean±SD)	9,31±3,17	8,37±2,14	0,160
Vitamin B12 Levels(pg/ml) (mean±SD)	415,46±158,89	366,25±163,02	0,204
NLR	1,48±0,58	1,74±0,95	0,157
PLR	121,96±39,14	142,79±47,48	<i>0,047</i>

Independent-samples t-test for continuous variables were used to test group differences.
 Italic font indicates statistical significance: $p < 0.05$
 Abbreviations: SD, Standard Deviation; SLD, Specific Learning Disorder; TSH, Thyroid Stimulating Hormone; WBC, White Blood Cell; NLR, Neutrophil lymphocyte ratio; PLR, Platelet lymphocyte ratio.

*Independent-samples t-test

When blood parameters were evaluated, no significant difference was found between the groups in terms of erythrocyte, neutrophil, platelet, T4, TSH, folic acid, vitamin B12, and NLR values. Lymphocyte count and WBC were significantly higher in the SLD group than in the control group ($p = 0.003$, $p = 0.006$, respectively). A significant difference was detected between the groups regarding PLR ($p = 0.047$). General characteristics and biochemical values of the groups are shown in Table 2. (Table 2)

Comparison of Parameters according to SLD Severity

When blood parameters were evaluated according to the severity of SLD, no significant difference was found between the groups in terms of erythrocyte, neutrophil, lymphocyte, WBC, platelet, T4, TSH, folic acid, vitamin B12, PLR, and NLR values. General characteristics and biochemical values of the groups are shown in Table 3. (Table 3)

Table 3. Comparison of parameters according to SLD severity

	Mild(n=7)	Moderate(n=13)	Severe (n=19)	p-value
Erythrocyte(10 ³ /uL) (mean±SD)	5,25±0,19	5,15±0,56	4,79±0,43	0,006
Neutrophil(10 ³ /uL) (mean±SD)	4,68±2,35	4,10±1,49	4,03±1,58	0,681
Lymphocyte(10 ³ /uL) (mean±SD)	3,42±1,14	2,70±1,07	2,97±0,76	0,281
WBC (10 ³ /uL) (mean±SD)	8,96±3,33	7,53±2,57	7,78±1,80	0,428
Platelet (10 ³ /uL) (mean±SD)	379,71±78,19	325,62±64,12	329,47±63,88	0,188
T4 (mcg/dl) (mean±SD)	1,33±0,08	1,31±0,23	1,25±0,13	0,425
TSH (mIU/L) (mean±SD)	2,67±1,34	2,10±0,45	2,32±1,11	0,481
Folic acid (ng/ml) (mean±SD)	9,74±1,82	10,26±4,53	8,49±2,23	0,286
Vitamin B12 Levels (pg/ml) (mean±SD)	445,57±209,86	412,38±161,618	406,47±144,08	0,860
NLR	1.34±0.35	1.64±0.62	1.43±0.62	0.478
PLR	120.54±47.42	132.70±48.05	115.14±28.52	0.470

One-way ANOVA for continuous variables were used to test group differences.
 Bold font indicates statistical significance: $p < 0.05$
 Abbreviations: SD, Standard Deviation; SLD, Specific Learning Disorder; TSH, Thyroid Stimulating Hormone; WBC, White Blood Cell; NLR, Neutrophil lymphocyte ratio; PLR, Platelet lymphocyte ratio.

Discussion

The present study sought to compare inflammatory markers and biochemical parameters between a specific learning disorder (SLD) group and a control group. The findings indicated that no notable discrepancy was observed between the two groups concerning erythrocyte, neutrophil, platelet, T4, TSH, folic acid,

vitamin B12, and NLR values. Nevertheless, the lymphocyte count and WBC values were observed to be markedly elevated in the SLD group in comparison to the control group. Additionally, a notable discrepancy was observed between the two groups about PLR.

The literature review highlighted a limited number of studies examining B12 and folic acid levels in individuals with SLD. These studies largely corroborate our findings.

For instance, Esnafoğlu's study reported no significant difference in vitamin B12 levels between SLD and control groups, although folic acid levels were notably lower in the SLD group [12]. Similarly, a 2016 study investigating B12 and zinc levels found no significant difference in B12 levels [13]. Deficiencies in vitamin B12 and folate have been associated with learning difficulties, psychosomatic symptoms, and anxiety in children with attention-deficit/hyperactivity disorder (ADHD) [14, 15]. In some cases, children with asymptomatic deficiencies in these nutrients may present with inattention or developmental delays, warranting an evaluation for potential neurodevelopmental disorders. Consequently, clinicians should consider assessing micronutrient levels when there is clinical suspicion of such deficiencies. The varying results observed in SLD, despite its classification as a neurodevelopmental disorder, may stem from differences in socioeconomic status and nutritional practices within the studied population.

Another parameter analyzed in this study was thyroid function. Thyroid hormones, including free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH), play a vital role in regulating motor, cognitive, and emotional processes. Extensive evidence underscores the adverse effects of maternal thyroid dysfunction on fetal brain development [17]. However, a review of the literature revealed no previous studies investigating thyroid function specifically in the context of SLD. In this study, thyroid function levels did not differ significantly between the SLD and control groups, potentially due to sample selection criteria or the limited sample size. Nonetheless, further research is necessary to clarify the role of thyroid function in brain development and its potential implications for SLD.

Systemic inflammation has been proposed as a potential contributor to the etiology of psychiatric disorders, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). However, the precise nature of the relationship between neurodevelopmental disorders and systemic inflammation remains uncertain [18]. In our study, an increase in white blood cell (WBC) and lymphocyte levels may serve as an indicator of chronic inflammation in individuals with learning disabilities. Chronic inflammation, particularly in neurodevelopmental disorders, can result in a state of constant stress in the brain and body. It is established that chronic inflammation has a deleterious impact on synaptic plasticity and neurodevelopment in the brain [19]. This may contribute to the etiology of cognitive disorders such as learning disabilities.

Secondly, individuals with learning disabilities may exhibit disturbances in the regulation of the immune system. An increase in white blood cell (WBC) and lymphocyte levels may indicate that the body's immune system is hyperactive. Such outcomes may be attributable to environmental factors, including infections or maternal immune activation, to which children with learning disabilities are exposed, particularly during prenatal or

early childhood. Maternal immune activation may result in the triggering of inflammatory responses in the fetus, which may subsequently give rise to neurodevelopmental consequences [20].

Also, a low platelet-to-lymphocyte ratio (PLR) may signify an alternative aspect of the immune response in individuals with learning disabilities. A low PLR indicates a reduction in platelet count relative to lymphocyte count, which may contribute to an increased complexity of the inflammatory state. Consequently, low platelet counts may result in a weakened or altered inflammatory response, which can occur in several ways [21]. A low PLR may provide insights into the general impact of the immune system in individuals with learning disabilities, and the effects of this condition on cognitive functions warrant further investigation.

In the study conducted by Bilac and colleagues, a total of 64 participants were included, comprising 31 children with SLD and 33 healthy children. The study yielded findings indicating that the patient group exhibited elevated neutrophil and lymphocyte counts and NLR values in comparison to the control group. PLR values did not differ significantly between the two groups. Nevertheless, regression analyses revealed that the NLR value, age, and gender, which were identified as independent variables, did not exert a significant influence on SLD [22]. In a further study conducted in 2023, no significant difference was observed between the two groups in terms of hemoglobin (Hb), red cell distribution width (RDW), platelet crit (PCT), platelet distribution width (PDW), white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR) and thrombocyte count (TLO) levels. The platelet count was observed to be higher in the SLD group. This study aimed to investigate the relationship between SLD types and to determine whether a significant difference exists. The results demonstrated that no significant difference was found [23]. Unlike earlier research, our study categorized SLD by severity but found no significant differences in blood parameters across severity levels.

While our findings provide valuable insights, the study has limitations, including a small sample size, potential confounding factors such as nutrition and physical activity, and a lack of direct measures of neuroinflammation. Future longitudinal studies with larger samples are needed to clarify the role of inflammation and immune system changes in SLD and their potential impact on cognitive development.

Conclusion

In conclusion, elevated white blood cell (WBC) and lymphocyte levels, along with a low platelet-to-lymphocyte ratio (PLR), may reflect chronic inflammation and immune system dysregulation in individuals with learning disabilities. These findings offer valuable insights into the potential link between immune responses and neurodevelopmental processes in this population. To build on this foundation, future research should address

current limitations by utilizing larger sample sizes, adopting longitudinal study designs, and accounting for potential confounding factors. Furthermore, investigating direct markers of neuroinflammation and exploring the mechanisms behind these hematological changes in SLD is essential. A deeper understanding of these processes could pave the way for targeted interventions aimed at reducing the impact of inflammation on cognitive and learning outcomes in children with SLD.

Conflict of interest

The author declares no conflict of interest, financial or otherwise.

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