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The investigation of the inflammation hypothesis in children diagnosed with ASD and ADHD using complete blood count variables

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Abstract

Objective: This study aims to compare the inflammation-related complete blood count (CBC) variables in children diagnosed with Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD).

Materials and Methods: Retrospective data on CBC values and Childhood Autism Rating Scale (CARS) scores for 30 children with ASD and 30 with ADHD, aged 60-108 months, were retrieved from hospital automation systems. Inflammatory-related CBC parameters, including hemoglobin (Hb), red cell distribution width (RDW), neutrophil, lymphocyte, monocyte, and platelet counts, mean cell volume (MCV), mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR), were compared between the two diagnostic groups. This study has a retrospective and cross-sectional design.

Results: Children with ASD exhibited significantly higher platelet counts ($p=0.003$; $t=3.052$; $d=0.788$) and PLR ($p = 0.044$; $Z = -2.011$; $r = 0.259$), whereas MPV was significantly lower ($p=0.029$; $t=-2.241$; $d=-0.579$) compared to children with ADHD. No significant differences were observed in other parameters. Additionally, a negative correlation was identified between CARS scores and RDW values in children with ASD ($p = 0.035$, $r = -0.387$).

Conclusion: Although ASD and ADHD are two neurodevelopmental disorders in which inflammation has been investigated in their pathophysiology, the inflammatory processes may differ between the two conditions. Future genetic and biochemical studies related to platelets in ASD may provide further insights into this area.

Keywords: autism, attention deficit hyperactivity disorder, inflammation, PLR, MPV

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) are two neurodevelopmental disorders. Although multifactorial genetic factors are believed to play a role in the etiology of both conditions, the importance of gene-environment interactions is also emphasized [1,2]. Environmental factors suggested to play a role in etiology include prenatal maternal inflammation, systemic inflammation in the individual, and neuroinflammation. Some studies have shown that individuals diagnosed with ASD and ADHD exhibit an increase in certain inflammation-related cytokines [3,4]. On the other hand, due to their cost-effectiveness and minimally invasive nature, analyzing certain inflammation-related parameters in peripheral blood has recently gained prominence. Various studies have demonstrated that parameters easily obtained from a complete blood count, such as neutrophil, monocyte, lymphocyte

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counts, and neutrophil/lymphocyte ratio (NLR), differ in neurodevelopmental disorders compared to healthy controls, similar to findings in cardiovascular diseases, cancer, rheumatological conditions, and other illnesses [5,6].

To date, no consistent results have been obtained regarding any parameters derived from complete blood count in children diagnosed with ADHD and ASD. Nevertheless, a significant increase in NLR has been reported in many studies for both disorders compared to controls [5,7]. However, studies also report no such difference, and some even report lower NLR levels [8-10]. In addition to NLR, variables such as neutrophil, lymphocyte, monocyte counts, mean platelet volume (MPV), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) have also been shown to differ in children diagnosed with ASD and ADHD compared to healthy controls [11,12].

Differences related to NLR are explained in terms of immune response and inflammation [13]. Additionally, the importance of platelet and platelet-related variables in neurodevelopmental disorders, compared to healthy controls, is highlighted [14,15]. For example, serotonin is emphasized as a significant shared product that functions as a neurotransmitter in the neuronal system, is secreted from the digestive system, and is stored in platelet granules. Several studies have reported increased serotonin levels in autism compared to healthy controls [16,17].

Variations in findings across previous studies on this topic may be attributed to differences in participant age groups, wide age ranges, and the cross-sectional nature of the measurements. In this study, based on the knowledge that blood cell parameters can vary with age, the age range was kept short [18]. Most literature compares individuals diagnosed with ASD and ADHD to healthy controls. However, studies directly comparing inflammation-related blood parameters between these two neurodevelopmental disorders are limited [5,19]. Despite both being neurodevelopmental disorders, considering the differences in their treatments, we hypothesize that these conditions may also differ in inflammation-related parameters. Additionally, based on past findings of platelet-related differences and hyperserotoninemia in ASD, we hypothesize that autism may involve a different inflammatory process than ADHD.

This study compares chronic inflammatory blood parameters in children aged 5–8 years with ASD and ADHD. We hypothesize that inflammation-related parameters will differ significantly between these disorders, with ASD patients displaying more prominent inflammatory markers than the ADHD group.

Materials and Methods

This study was designed as a retrospective, cross-sectional, and comparative analysis of children aged 60 to 108 months who were diagnosed with ASD and ADHD at Hacettepe University's Pediatric and Adolescent Psychiatry outpatient clinic between January 1, 2023, and January 1, 2025. Data collection was subsequently performed between September 15 and September 30, 2025, by screening the hospital's electronic automation system. The Childhood Autism Rating Scale (CARS) scores obtained during psychiatric assessment and complete blood count (CBC) results obtained by the pediatrician were also retrieved from the hospital automation system.

The diagnoses of ASD and ADHD were made according to DSM-5 criteria during psychiatric evaluations. In the hospital automation screening, only patients with ASD and ADHD diagnoses who did not have a secondary psychiatric diagnosis were included in the study. Furthermore, participants were not using any psychotropic medication before. Patients with epilepsy, intellectual disability, organic brain injury, psychotic disorders, or acute or chronic physical illnesses, as well as those exhibiting abnormalities in CBC indicative of specific medical conditions, were excluded from the study.

CBC parameters, including hemoglobin (Hb), red cell distribution width (RDW), neutrophil count, lymphocyte count, monocyte count, platelet count, mean cell volume (MCV), mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR), were obtained.

The statistical analysis was performed using SPSS (version 31). The assumption of normality for numerical variables was examined using the Shapiro–Wilk goodness-of-fit test. Homogeneity of variances was analyzed using Levene's test. For numerical data, the independent two-sample t-test was used for normally

distributed variables, while the Mann–Whitney U test was used for non-normally distributed variables. Categorical variables are presented as numbers and percentages. The Pearson Chi-Square test was employed to analyze categorical variables. To evaluate the relationship between two continuous variables, Pearson's correlation was used for normally distributed variables, and Spearman's correlation was used for non-normally distributed variables. Effect sizes were calculated for each statistical test to assess the clinical significance of differences between the two means. The level of statistical significance was set at $p<0.05$.

Ethical approval for study number SBA 25/883 was granted by the Hacettepe University Health Sciences Research Ethics Committee on November 11, 2025, with decision number 2025/21-33.

Childhood Autism Rating Scale (CARS)

The CARS consists of 15 items, each rated on a half-point scale from 1 to 4. The scale is used to distinguish children on the autism spectrum from those with intellectual disabilities and to assess the severity of autism. It provides an objective and measurable evaluation based on direct observation of behaviors. The scale can be applied to children of all age groups, from preschool age onwards. Scores on the scale range from 15 to 60 points. Children who score below 30 do not exhibit autistic signs, those with scores between 30 and 36.5 are considered to have mild to moderate impairment, and those with scores from 37 to 60 are classified as having severe impairment [20]. The scale has been validated for Turkish language and culture, with studies demonstrating good validity and reliability [21].

Results

There was no significant difference in age and gender distribution between the ADHD and ASD groups ($p>0.05$). In the ADHD group, 27 males (90%) and 3 females (10%) were included, whereas in the ASD group, 23 males (76.7%) and 7 females (23.3%) were included. The median age in the ADHD group was 70.00 months (IQR: 14.75), whereas in the ASD group, the median age was 72.50 months (IQR: 15.75). The mean CARS score in the ASD group was 35.23 ± 5.24.

In this study, significant differences between the ASD and ADHD groups were observed exclusively in platelet-related parameters, specifically PLR, MPV, and platelet count. The median PLR was higher in the ASD group compared to the ADHD group [102.79 (48.10) vs. 84.62 (43.60)]. Similarly, the mean platelet count was significantly higher in children with ASD (349.80 ± 71.18) than in those with ADHD (295.73 ± 65.95). Conversely, the mean MPV was lower in the ASD group (7.72 ± 0.64) than in the ADHD group (8.14 ± 0.80). Statistical analysis confirmed significant differences for PLR (**p=0.044**, Z=-2.011, r=0.259), MPV (**p=0.029**, t=-2.241, d=-0.579), and platelet count (**p=0.003**, t=3.052, d=0.788). No other variables showed significant intergroup differences. Detailed comparisons of the complete blood profiles are presented in Table 1.

No significant correlation was found between CARS scores and variables such as hemoglobin, platelet count, neutrophil, lymphocyte, monocyte, MCV, MPV, NLR, MLR, and PLR among children with ASD ($p>0.05$). A moderate effect size negative correlation was observed between CARS scores and RDW (**p=0.035**, r=-0.387).

Discussion

In this study, the inflammatory markers in two neurodevelopmental disorders, ADHD and ASD, were investigated in children aged 5-8 years using complete blood count parameters, such as NLR and PLR, which have been reported in the literature as potentially useful for inflammation monitoring [5,13]. The results showed that children with ASD had higher platelet count and PLR values, and lower MPV values compared to children with ADHD. No significant differences were found between the two groups for other variables.

A significant portion of existing studies have reported that NLR and PLR ratios are significantly increased in patients diagnosed with both ASD and ADHD compared to healthy controls [7,22]. However, although the number of such studies is limited, some research has also shown that this difference in NLR or PLR is not observed in at least one of the two disorders [10,23]. Additionally, other studies have demonstrated that MLR, neutrophil count, and monocyte count are also higher in individuals with ADHD and ASD compared to healthy controls [12,24]. There are studies that report significantly higher platelet counts in both disorders

compared to healthy controls, as well as studies that report no significant difference [11,16,25-27].

The neuroinflammation hypothesis is one of the possible explanations proposed in the pathophysiology of ASD and ADHD, and many studies continue to investigate this area. For example, it has been suggested that cytokines such as interleukin-1β (IL-1β), interleukin-2 (IL-2), and interleukin-6 (IL-6) may play a role in ADHD, while cytokines like IL-6 and tumor necrosis factor alpha (TNF-alpha) may be involved in ASD [28,29]. The role of neutrophils in acute inflammation is already well known. It has been increasingly understood in recent decades that they also play an important role in chronic inflammation. The role of neutrophils has been demonstrated in many chronic diseases, including autoimmune and cardiovascular diseases [30]. For this reason, it is known that NLR ratios increase in various chronic diseases and are used to predict disease outcomes [6]. In studies on ADHD and ASD, a significant portion has shown that NLR values are higher in affected individuals compared to healthy controls [5,7]. However, some studies have reported no difference or lower values [8,10].

In our study, the lack of difference in NLR ratios between the two patient groups could be due to several factors. Although patients without additional psychiatric diagnoses were included based on hospital records, the absence of a structured diagnostic tool means there could be unidentified psychiatric comorbidities, such as tics and depression, which can affect NLR ratios [31,32]. Another possibility is that children with ASD may have been diagnosed and started treatment before the age of 5-8 years, leading to a decrease in ASD severity. Indeed, the relatively low mean CARS scores in children with ASD suggest that the severity of their condition is not high. Perhaps the interventions used for autism treatment have reduced inflammation severity neurobiologically through epigenetic mechanisms [33]. Longitudinal studies monitoring inflammation markers in individuals diagnosed with ASD and ADHD would be valuable to test these hypotheses.

In our study, we primarily found that variables related to platelets differ in children diagnosed with ASD and ADHD. More specifically, we observed that children with ASD had higher platelet counts and PLR values, while their MPV was lower. There are studies showing increased platelet counts and PLR values in studies involving children with both ASD and ADHD [16,23,25,34]. MPV levels are generally higher in children diagnosed with ADHD compared to healthy controls [11,27]. On the other hand, in studies involving children with ASD,

Table 1. Comparison of complete blood count parameters in children diagnosed with ASD and ADHD

	ASD	ADHD	Test statistic	p-value	Effect size
	Mean±SD Median (IQR)	Mean±SD Median (IQR)			
Hb (g/dL)	12.94±1.00	12.75±0.96	0.759^{t}	.451	0.196^{d}
RDW (%)	13.60 (1.70)	14.15 (1.01)	-1.450^{z}	.147	-0.187^{r}
Neutrophil (10^3/ul)	3.53±1.14	3.85±1.37	-0.990^{t}	.327	-0.256^{d}
Lymphocyte (10^3/ul)	3.56 (1.25)	3.55 (1.60)	-0.370^{z}	.711	-0.047^{r}
Monocyte (10^3/ul)	0.60±0.33	0.66±0.20	-0.404^{z}	.686	-0.052^{r}
Platelet (10^3/ul)	349.80±71.18	295.73±65.95	3.052^{t}	**.003***	0.788^{d}
MCV (fL)	79.30 (7.00)	78.25 (5.22)	-0.614^{z}	.539	-0.079^{r}
MPV (fL)	7.72±0.64	8.14±0.80	-2.241^{t}	**.029***	-0.579^{d}
NLR	1.06±0.51	1.20±0.66	-0.939^{t}	.352	-0.243^{d}
MLR	0.18 (0.08)	0.17 (0.15)	-0.274^{z}	.784	-0.035^{r}
PLR	102.79 (48.10)	84.62 (43.60)	-2.011^{z}	**.044***	-0.259^{r}

ASD: autism spectrum disorder; ADHD: attention deficit hyperactivity disorder; Hb: hemoglobin; RDW: red cell distribution width; MCV: mean cell volume; MPV: mean platelet volume, NLR: neutrophil/lymphocyte ratio; MLR: monocyte/lymphocyte ratio; PLR: platelet/lymphocyte ratio; Z: Z test value; t: t test value; d: cohen's d value; r: effect size value; *p<0.05.

MPV values have been found to be lower than those of healthy controls or show no significant difference [14,35]. The finding of lower MPV in children with ASD may reflect the production of smaller platelets following platelet activation induced by chronic inflammation. As such, the decrease in MPV should be interpreted not as a direct biomarker of ASD, but rather as a correlate of platelet activation dynamics during inflammation [36].

Studies related to platelet products in individuals with ASD are not entirely new [37,38]. Some studies have reported various abnormalities in platelet function related to ASD [14]. For example, these studies have suggested that there may be a relationship between hyperserotoninemia detected in the blood of ASD patients and platelet serotonin secretion, as well as overlaps in certain genetic expression profiles of neurons and platelets, which use similar signaling pathways [15,17]. On the other hand, platelets play an important role in inflammation together with neutrophils and influence each other's functions [39]. All these reasons highlight the need for further research investigating the relationship between platelet functions and ASD, especially in inflammation-related studies, at the genetic and biochemical levels.

The finding of a negative correlation between CARS scores and RDW in children diagnosed with ASD was contrary to expectations. In the literature, RDW values in children with ASD are generally reported to be similar to those of healthy children. Higher RDW values in ASD are typically associated with nutritional deficiencies resulting from selective eating behaviors or chronic inflammation. However, a low RDW value is not considered a pathological indicator; rather, it reflects a more uniform distribution of erythrocytes in the blood [40]. These unexpected results may be linked to variations in nutritional status, erythropoietic stability, or oxidative stress among participants with ASD [40]. It is also probable that measurement variability, along with our reliance on retrospective records and a relatively small cohort, influenced these outcomes.

Our study has some limitations. These include the retrospective collection of data from hospital records, which may lead to the omission of possible psychiatric comorbidities, the absence of a healthy control group, and the relatively small sample size due to the narrow age range of participants. Furthermore, because the study is based on retrospective data and lacks longitudinal follow-up, the findings should be interpreted as cross-sectional rather than causal. Even though our sample was limited to children without acute or chronic illnesses and no history of psychotropic use, inflammatory markers could still have been influenced by past medications, subclinical infections, or nutritional shifts. Not being able to control for these factors remains a limitation of this research.

Among the strengths of the study, it is noted that, given that hematological values vary across age groups, the age range of participants was kept narrow to ensure homogeneity. Additionally, instead of using a single inflammation marker such as NLR, multiple parameters were employed. Furthermore, the study compared inflammation markers in two neurodevelopmental disorders believed to contribute to neuroinflammation.

Conclusion

Although previous studies have shown that inflammation may play a role in both ASD and ADHD diagnoses, the number of studies comparing these two disorders is limited, and therefore, our study is expected to contribute to the literature. In this study, children diagnosed with ASD had significantly higher platelet counts and PLR values compared to children with ADHD, while MPV was found to be significantly lower. Based on our findings, we recommend conducting genetic and biochemical studies involving platelet-related parameters in inflammation research in ASD.

Author contributions

Conception and design: C.A., K.N., D.Ü.; Data acquisition: C.A., K.N., D.Ü., B.K.; Data analysis: C.A., B.K.; Data interpretation: C.A., K.N., D.Ü., B.K.; Drafting of the manuscript: C.A., B.K.; Critical revision of the manuscript: C.A., K.N., D.Ü.. All authors reviewed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of this study.

Ethical approval

This study was approved by the Hacettepe University Health Sciences Research Ethics Committee (Date: November 11, 2025, Decision/Protocol No: 2025/21-33). Ethics committee approval and informed consent were not required for this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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