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Novel low-grade inflammation markers in children with attention deficit hyperactivity disorder

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ABSTRACT

Aims: The course of low-grade inflammation markers, including platelet-monocyte ratio (PMR), mean platelet volume-lymphocyte ratio (MPVLR), and the mean platelet volume-platelet ratio (MPVPR), is not known in children with Attention Deficit Hyperactivity Disorder (ADHD). The primary objective of this study was to compare PMR, MPVLR, and MPVPR in children with ADHD versus healthy controls.

Methods: A retrospective, case-control, cross-sectional study was performed with children aged 6-18 years with ADHD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. An age, gender, and body mass index-matched healthy control group was formed. Platelet-lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), basophil-lymphocyte ratio (BLR), PMR, MPVLR, MPVPR, and systemic immunity-inflammation index (SII) were compared between the two groups.

Results: The study included 79 children with ADHD and 70 controls. NLR, BLR, SII, PMR, and MPVPR were not significantly different between the two groups. However, the PLR was higher in the ADHD group than in the control group ($p<0.001$). Additionally, the MLR ($p=0.048$) and the MPVLR ($p=0.018$) were higher in the ADHD group than in the control group. Receiver operating characteristic analysis demonstrated that the MPVLR level was not significantly associated with predicting the presence of ADHD [area under the curve (AUC): 0.58, $p=0.077$]. However, PLR (AUC: 0.68, $p<0.001$) and MLR (AUC: 0.59, $p=0.048$) values showed predictive potential for ADHD, with cut-off values of 110.23 and 0.19, respectively.

Conclusions: Of the variables evaluated, significant increases in PLR and MLR support the hypothesis that an altered inflammatory response may be associated with ADHD. Further research is needed to confirm these findings in prospectively designed studies.

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most widespread neurodevelopmental disorders that affect the lives of children and adolescents (1). The global prevalence of ADHD is approximately 5.3% among children of school age, exhibiting a variability that spans from 2.5% to 12.5% (2). Characterized by inattention, hyperactivity, and impulsivity, ADHD not only poses significant challenges for affected individuals but also places a substantial burden on their families and society (3). ADHD symptoms are not confined to childhood; they persist into adulthood at a rate ranging from 15% to 80% (4,5). Additionally, individuals with ADHD may often experience comorbid psychiatric disorders, including learning disorders, oppositional defiant disorder, conduct disorder, depression, and anxiety. ADHD and its comorbid conditions can result in significant social, academic, and psychological challenges in all stages of child and adolescent development (3). While extensive research has explored the neurobiological and genetic underpinnings of ADHD, the involvement of immune system dysregulation and inflammatory processes in the pathogenesis of this condition has received increasing attention in recent years (6).

White blood cells (WBCs), including lymphocytes, basophils, monocytes, and neutrophils, along with parameters such as mean platelet volume (MPV) and red blood cell distribution width (RDW), in conjunction with various derived ratios, such as the platelet-lymphocyte ratio (PLR), monocyte-lymphocyte ratio (MLR), neutrophil-lymphocyte ratio (NLR), basophil-lymphocyte ratio (BLR), and the systemic immunity-inflammation index (SII), have emerged as indicative biomarkers of systemic inflammation (7-10). These markers are derived from the complete blood count (CBC) and offer a cost-effective means of assessing an individual's inflammatory status. Furthermore, novel prognostic indicators, including the platelet-monocyte ratio (PMR), MPV-to-platelet ratio (MPVPR), and MPV-lymphocyte ratio (MPVLR), have shown promise as innovative predictors of cardiovascular disorders and seizures (11-13).

Neuroinflammation, characterized by the activation of immune cells within the central nervous system, has been implicated in various neuropsychiatric conditions, including ADHD (14). Emerging evidence suggests that chronic inflammation may disrupt neurodevelopmental processes, alter neurotransmitter systems, and impair cognitive functions, all of which may contribute to ADHD symptomatology (15,16). Empirical findings indicate a heightened incidence of ADHD in individuals with rheumatic disorders (17). However, the relationship between low-grade inflammation markers and ADHD in pediatric populations remains an area of ongoing investigation (14).

A study on the relationship between ADHD and hematological parameters revealed elevated WBC counts, neutrophil counts, MPV, PLR, NLR, and PDW in individuals with ADHD compared with healthy controls (18). Different authors have also reported higher MPV levels in individuals with ADHD (19,20). A recent study showed increased PLR and NLR in adolescents with ADHD compared with healthy controls (21). Another study involving children with ADHD in Turkey showed statistically significant increases in NLR, PLR, MLR, MPV and neutrophil counts and lower lymphocyte counts in individuals with ADHD compared to controls (22). On the other hand, several studies have found no significant differences in CBC parameters in individuals with ADHD (23,24).

The above mentioned inconsistent findings are noteworthy, and the potential roles of PMR, MPVLR, and MPVPR as novel prognostic indicators in children with ADHD remain unknown. Therefore, the current study aimed to examine the circulating inflammation markers, including NLR, PLR, MLR, BLR, PMR, MPVLR, MPVPR, and SII in a pediatric cohort of patients with ADHD diagnosis and healthy controls.

Methods

Participants and procedures

This retrospective, cross-sectional, case-control study included children of 6 to 18 years admitted to the child psychiatry outpatient clinic at Başkent University Faculty of Medicine between August 2022 and August 2023. The patients met the diagnostic criteria for ADHD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. The exclusion criteria were having received previous treatment for ADHD, having psychiatric comorbidities (mental retardation, learning disability, autism, psychosis, depression, anxiety disorders or bipolar affective disorder), having a chronic medical illness (such as autoimmune disease, allergic disease, hypertension, diabetes mellitus, etc.), acute or chronic infectious diseases, and having CBC values outside the normal limits [anemia (hemoglobin <11 g/dL), leukocytosis (>10,000/μL) or leukopenia (<4000/μL), thrombocytopenia (<150.000 mm^3/dL), or thrombocytosis (>400.000 mm^3/dL)], obesity (at or above the 95th percentile for age and gender), or the use of any drugs, substances, or cigarettes. The control group consisted of individuals with no known acute or chronic physical or psychiatric diseases and who were admitted for a routine check-up at the pediatric outpatient clinic on similar dates. They were matched with children with ADHD for gender, age, and body mass index (BMI). The study was approved by Başkent University, Medical and Health Sciences Research and Ethics Committee (project number: KA23/313, date: 19.09.2021). The procedures conformed to the principles of the Declaration of Helsinki revised in 2013.

All data were obtained from hospital records, including age, gender, body weight, height, BMI, and hematological parameters (leukocyte, thrombocyte, neutrophil, lymphocyte, monocyte, basophil count, RDW, MPV). Subsequently, NLR (absolute neutrophil count/absolute lymphocyte count), PLR (absolute platelet count/absolute lymphocyte count), MLR (absolute monocyte count/absolute lymphocyte count), BLR (absolute basophil count/absolute lymphocyte count), SII (platelet count × neutrophil count/lymphocyte count), PMR (absolute platelet count/absolute monocyte count), MPVLR (mean platelet volume/absolute lymphocyte count), and MPVPR (mean platelet volume/absolute platelet count) were calculated.

Statistical Analysis

A priori power analysis for sample size calculation was conducted using G Power analysis (25), which determined that a minimum of 64 participants per group was required, based on a two-tailed t-test for means (effect size d=0.50, α=0.05, 1-β=0.80). Data analysis was performed using Statistical Package for Social Sciences 25.0 (Armonk, New York: IBM corporation).

The normality of distribution was tested using the Shapiro-Wilk test, histogram plots, mean-median values, skewness, and kurtosis statistics. The chi-square test was used to compare categorical variables, and the Student's t-test was used to compare continuous variables showing normal distribution. The Mann-Whitney U test was used to compare non-normally distributed continuous variables. Pearson correlation analysis was used to check the correlations between age, gender, BMI, and inflammation markers. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic thresholds of serum inflammatory marker levels. P<0.05 was considered statistically significant.

Results

Of the 168 ADHD diagnoses, 89 subjects were excluded, primarily because of psychiatric comorbidities (62%). The ADHD group included 79 individuals with a mean age of 10.35 years [standard deviation (SD)=3.22] and male predominance (69.6%). The control group also included 79 individuals, with a mean age of 10.64 years (SD=3.63) and 72.9% male predominance. Age, gender, and BMI were not significantly different between the two groups (Table 1).

Table 1. Comparison of the groups in terms of age, gender, and BMI

Variables	ADHD (n=79)	Controls (n=70)	p
Age (years)*	10.35±3.22	10.64±3.63	0.613[a]
Gender**			
Female	24 (30.4)	19 (27.1)	0.663[b]
Male	55 (69.6)	51 (72.9)	
BMI (kg/m^2)*	19.88±4.91	19.10±2.47	0.215[a]

Data are expressed as *mean±standard deviation or **number (%).
[a]Independent sample t-test was used, [b]Pearson's chi-squared test was used.
ADHD: Attention-deficit/hyperactivity disorder, BMI: Body mass index

Age displayed statistically significant positive correlations with MPV, MPVLR, and MPVPR values (r=0.282, p<0.001; r=0.391, p<0.001; r=0.393, p<0.001, respectively), and inverse correlations with platelet count, lymphocyte count, and basophil count (r=-0.301, p<0.001; r=-0.337, p<0.001; r=-0.215, p=0.008, respectively). No significant correlations were found between age and RDW, neutrophils, monocytes, NLR, PLR, MLR, BLR, PMR, or SII levels.

The PLR was significantly higher in the ADHD group than in the control group [123.8 (SD=37.5) vs. 103.0 (SD=31.1), p<0.001]. Additionally, the median MLR [0.20 (IQR=0.08) vs. 0.19 (IQR=0.07), p=0.048], and the median MPVLR [3.2 (IQR=1.2) vs. 2.8 (IQR=0.7), p=0.018] were higher in the ADHD group than in the control group. However, there were no statistically significant between-group differences in leukocyte, platelet, neutrophil, lymphocyte, monocyte and basophil counts, RDW, and MPV (Table 2). In the ADHD group, NLR, SII, and PMR were higher (Table 2), but the differences did not reach statistical significance (p>0.05).

Serum MPVLR level was a predictor of ADHD [AUC: 0.58, 95% confidence interval (CI): 0.49-0.68, p=0.077]. On the other hand, PLR (AUC: 0.68, 95% CI: 0.59-0.77, p<0.001) and MLR (AUC: 0.59, 95% CI: 0.50-0.69, p=0.048) exhibited predictive

Table 2. Comparison of the groups in terms of laboratory parameters

Laboratory parameters	ADHD (n=79) Mean±SD/Median (IQR)	Controls (n=70) Mean±SD/Median (IQR)	p
White blood cell count (10^3/mm^3)	7.2±1.9	7.6±1.58	0.202[a]
Platelet count (10^9/L)	312.4±71.1	291.2±65.1	0.061[a]
Red blood cell distribution width (%)	12.2±1.5	12.5±1.1	0.182[a]
Mean platelet volume (fL)	7.7±1.3	7.8±1.1	0.517[a]
Neutrophils count (10^3/mm^3)	3.7±1.4	3.8±1.3	0.606[a]
Lymphocyte count (10^3/mm^3)	2.7±0.9	2.9±0.6	0.077[a]
Monocytes count (10^3/mm^3)	0.6±0.2	0.6±0.1	0.836[a]
Basophils count (10^3/mm^3)	0.05 (0.05)	0.06 (0.04)	0.400[b]
Neutrophil-lymphocyte ratio	1.5±0.7	1.4±0.6	0.244[a]
Platelet-lymphocyte ratio	123.8±37.5	103.0±31.1	**<0.001**[a]
Monocyte-lymphocyte ratio	0.20 (0.08)	0.19 (0.07)	**0.048**[b]
Basophil-lymphocyte ratio	0.02±0.01	0.02±0.01	0.732[a]
Systemic immunity-inflammation index	389.9 (276.5)	337.5 (200.9)	0.109[b]
Platelet-monocyte ratio	524.5 (292.5)	499.8 (220.92)	0.272[b]
MPV-lymphocyte ratio	3.2±1.2	2.8±0.7	**0.018**[a]
MPV-to-platelet ratio	0.03±0.01	0.03±0.01	0.087[a]

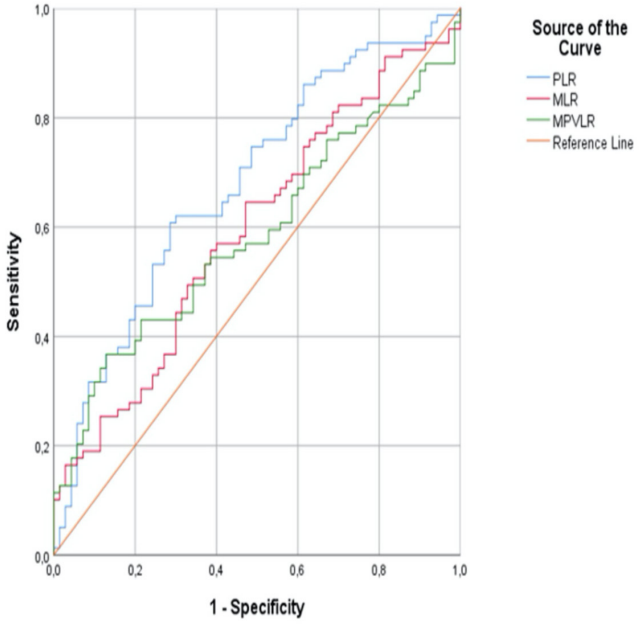
[a]: Independent sample t-test was used, and [b]:Mann-Whitney U-test was used. Bold values are significant.
SD: Standard deviation, IQR: Interquartile range, ADHD: Attention-deficit/hyperactivity disorder

value for diagnosing ADHD in ROC analysis. The recommended threshold value for PLR was 110.23, with a sensitivity of 62.9%, specificity of 70%, positive predictive value of 70%, and negative predictive value of 62%. A threshold of 0.19 was calculated for MLR, with a sensitivity of 64.6% and a specificity of 52.9% (Figure 1).

Discussion

Low-grade inflammation has been an investigation field concerning the underlying mechanisms and potential biomarkers associated with ADHD (26). One of the key features of this study is its pioneering evaluation of the relationship between PMR, MPVLR, and MPVPR with ADHD. Additionally, it is one of the rare studies in Turkey that simultaneously assessed multiple inflammation markers in children with ADHD. Based on the ROC analysis results, serum PLR and MLR have emerged as potential diagnostic markers for ADHD.

AUC values of 0.68 for PLR and 0.59 for MLR indicate moderate and fair discriminative abilities, respectively. While the AUC for PLR signifies a higher degree of discriminatory power, both ratios show statistical significance in differentiating between individuals with and without ADHD. It should be emphasized that the diagnostic capacity of PLR appears particularly promising, with an AUC well above the 0.5 threshold commonly associated with random chance. Previous studies have reported a higher predictive power of PLR than MLR, which is consistent with the current results (22,27).



Test variables	Area	p value	95% confidence interval	
			Lower bound	Upper bound
PLR	0.680	<0.001	0.594	0.765
MLR	0.594	0.048	0.503	0.685
MPVLR	0.584	0.077	0.492	0.676

Figure 1. ROC curve analysis for PLR, MLR, and MPVLR to discriminate ADHD and healthy control

PLR: Platelet-lymphocyte ratio, MLR: Monocyte-lymphocyte ratio, MPVLR: MPV-lymphocyte ratio, ADHD: Attention Deficit Hyperactivity Disorder

The current results align with prior research that indicated a relationship between PLR and ADHD (21,22) and between MLR and ADHD (22,27). In addition to leukocytes, platelets play a crucial role in inflammation. Similar to various disorders characterized by inflammation, ADHD has been associated with elevated levels of MPV and platelets (19,20). In our study, both groups showed MPV levels, platelet count, and lymphocyte counts within the normal range, with no significant between-group differences. However, a notably elevated PLR was observed within the ADHD group, emphasizing the relevance of this inflammatory ratio in the context of ADHD. However, previous studies have reported that these inflammation markers could not predict specific ADHD subtypes (22,27,28) or the severity of the condition (21,22,28). Moreover, Önder et al. (21) reported no statistically significant differences in the levels of inflammatory markers (NLR and PLR) between patients with and without psychopharmacological treatment for ADHD. Therefore, higher levels of low-grade inflammation markers, including PLR, may indicate ADHD. However, inflammation at a certain level or above may not be related to the severity or subtype of the disease.

While ADHD occurs due to various environmental causes in the presence of a genetic predisposition, the neurobiological basis of these interactions has not been elucidated (29). Brain development in neurodevelopmental disorders such as ADHD is most sensitive to environmental factors during the early stages (30). A cohort of over 1,500 preterm and low birth weight newborns showed that infants with elevated serum inflammation-related proteins during the first 2 postnatal weeks exhibited more attention problems at 2 years of age (31). Similarly, a prospective follow-up study determined that an increased systemic inflammatory response during the first month of life in children with a history of preterm birth was associated with a higher risk of ADHD (32). Many environmental risk factors are known to play a role in the etiology of ADHD (33), and ADHD seems to be associated with the inflammatory response, at least in a subgroup (23).

One inherent limitation of this investigation is its cross-sectional and retrospective design, which hinders causal relationships. Moreover, although the diagnostic accuracy of PLR and MLR is promising, they may not be sufficient standalone tools for diagnosing ADHD.

Conclusion

In summary, PLR and MLR may offer a cost-effective and easily accessible tool for the early detection of children

with ADHD. Future research may investigate the underlying biological mechanisms and validate our findings across diverse populations to establish broader clinical applicability. Ultimately, a multidimensional approach that integrates clinical evaluation, neuroimaging, and inflammation markers may provide a more precise and comprehensive diagnostic framework for ADHD.

Ethics

Ethics Committee Approval: The study was approved by Başkent University, Medical and Health Sciences Research and Ethics Committee (project number: KA23/313, date: 19.09.2021).

Informed Consent: Retrospective study.

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