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Original Article

High neutrophil to lymphocyte ratio and low mean platelet volume level in autism spectrum disorders

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Abstract

Aim: A growing number of studies have indicated that ASD is associated with neuro-inflammation. Recent research has indicated that complete blood count (CBC) may be used for assessing inflammatory markers, offering a cheap, simple and reproducible alternative that is also suitable for routine application. In this study, we evaluated complete blood count (CBC) data in children with ASD, aiming to explore associations between ASD and inflammation

Material and Methods: Patients diagnosed with ASD between 01.01.2018 and 31.08.2019 were included in the study. CBC results of patients from their initial visit to the clinic were analyzed. The control group consisted of children with matching age and gender visiting the clinic for general examination. CBC parameters of both the study and control groups were evaluated statistically.

Results: 67 children diagnosed with ASD and 67 healthy children were included in the study. In the ASD group, the neutrophil/lymphocyte ratio (NLR) was significantly higher than in the control group ($p<0.001$). The cut-off value for NLR was determined as 2.32. The levels of mean platelet volume (MPV) were significantly lower in the ASD group compared to the control group ($p<0.001$). The cut-off value for MPV was determined as 11.75 fl.

Conclusion: NLR and MPV measurements, which are simple and readily-available laboratory tests, may serve as important biomarkers for diagnosis of ASD. Increased NLR and lowered MPV levels may be used as screening tools and early intervention predictors for ASD. Further research with a larger sample size may clarify the involvement of inflammation in ASD.

Keywords: Autism spectrum disorder, inflammation, neutrophil/lymphocyte ratio.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with communication and social interaction problems. One in every 59 children is diagnosed with ASD and this prevalence is rising (1). Etiopathogenesis of ASD, while continuously studied, is not completely understood. Early diagnosis of ASD is of utmost importance, since brain development is the fastest during ages 0-5 and ASD directly affects learning ability of children (2). Connections between neurons in children with ASD are fewer in number and lower in quality. Regular stimulation especially during the early childhood period is necessary for healthy development of the brain and nervous system. Considering the importance of early diagnosis of ASD, the search for a simple biomarker suitable for routine application is an ongoing effort (3).

A growing number of studies have indicated that ASD is associated with neuro-inflammation and neuro-immune system disorders. Children with autism displayed higher inflammatory activity as shown by pro-inflammatory biomarker analysis. It has been reported that both Th1 and Th2 cytokines are more abundant in children with ASD. Values of IL-1β,IL-6,IL-17,IL-12p40,IL-12p70,S100B and TNF-α are considered as diagnostic tests of ASD in children (4). Additionally, maternal inflammation in particular has been shown to affect fetal brain development. There are also studies suggesting that central and peripheral inflammation in ASD patients persists through lifetime. However, methods of immunologic cell analysis for the diagnosis of ASD are considerably expensive, complex

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and hard to replicate (4,5).

Recent research has indicated that complete blood count (CBC) may be used for assessing inflammatory markers, offering a cheap, simple and reproducible alternative that is also suitable for routine application. CBC provides insight into the inflammatory state by indicating the cellular composition of blood, such as erythrocytes, neutrophils, lymphocytes, monocytes and platelets, as well as certain cellular ratios, such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) (6).

The foundation to our study was shaped by the facts that ASD is affected by many factors such as environment, genetic makeup and diet, and that the immune response in each patient is different (7,8). In our study, we aimed to determine whether CBC, which is a simple and readily-available laboratory test, can be utilized as an indicator of ASD etiopathogenesis, as well as an diagnosis tool for ASD.

MATERIAL and METHODS

In this retrospective study, we analyzed the CBC parameters of first-time patients visiting Health Sciences University Antalya Training and Research Hospital (Antalya, Turkey) diagnosed with ASD and those of first-time patients with matching age and gender visiting the clinic for general examination between 01.01.2018 and 31.08.2019. After retrospective screening, CBC data were evaluated statistically. The exclusion criteria were diagnoses of infection, and allergic and neoplastic/leukoproliferative disorders at the time of CBC sampling.

Statistical Analysis

Data were evaluated using the statistical analysis package of SPSS (version 15.0; IBM, Chicago, IL, USA). Results were given as mean ± standard deviation. Chi-square and Mann-Whitney U tests were performed to analyze data. Correlation between continuous variables was assessed with Pearson and Spearman correlation coefficients. Statistical significance between categorical variables were evaluated with either chi-square test or Fisher's exact test. p values of <0.05 were deemed as significant. Receiver operating characteristics (ROC) curve analysis was used for determining cut-off values.

RESULTS

Of the 67 ASD patients included in the study, 15 were female (23%) and 52 were male (77%). The ages within the ASD group ranged between 5 and 11, while the mean age was 7.78±3.60. 20(30%) of the 67 control patients were female and 47(70%) were male. Age within the control group varied between 6 and 10, with a mean of 7.90±2.70. Age and gender were not statistically different between the ASD and control groups. NLR values were significantly higher and MPV levels were significantly lower in the ASD group compared to the control group. No statistically significant difference was detected between the two groups in PLR, platelet/larger cell ratio (PLCR), monocyte count or platelet count (Table 1).

The cut-off value for NLR was found to be 2.32. ROC analysis determined the area under the curve (AUC) as 0.917 and p as 0.0001 (Figures 1&2), indicating highly sensitive and significant results. The cut-off value for MPV was 11.75 fL. In ROC analysis, AUC was 0.743 and p was 0.0001 (Figures 3,4), similarly indicating highly sensitive and significant results.

Table 1. CBC parameters of ASD patients and the control group

	ASD Patients (n=67)	Control Group (n=67)	P
Age (years)	7.78 ± 3.60	7.90 ± 2.70	0.813
Neutrophil (103/mm3)	4.95 ± 2.49	3.86 ± 2.76	0.008
Lymphocyte (103/mm3)	3.26 ± 1.30	3.10 ± 1.02	0.213
NLR	1.69 ± 0.81	1.13± 0.64	0.0001
PLR	112.76 ± 45	115.64 ± 40.45	0.342
MPV (fL)	8.13 ± 1.07	10.01 ± 0.82	0.0001
Platelet (103/mm3)	340.56 ± 105.48	331.46 ± 87.03	0.591

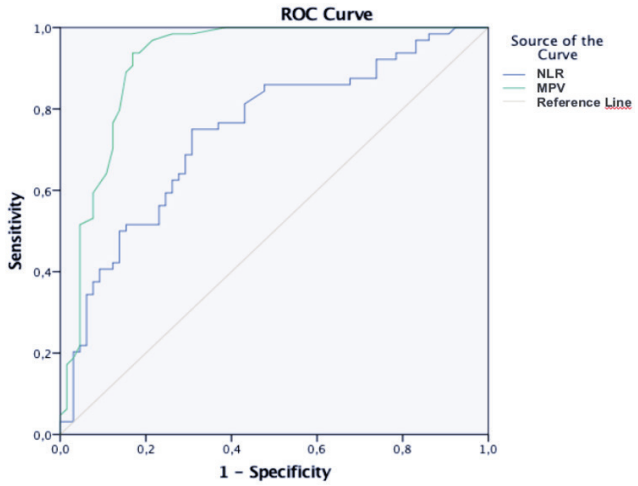

Figure 1. ROC analysis of NLR and MPV values

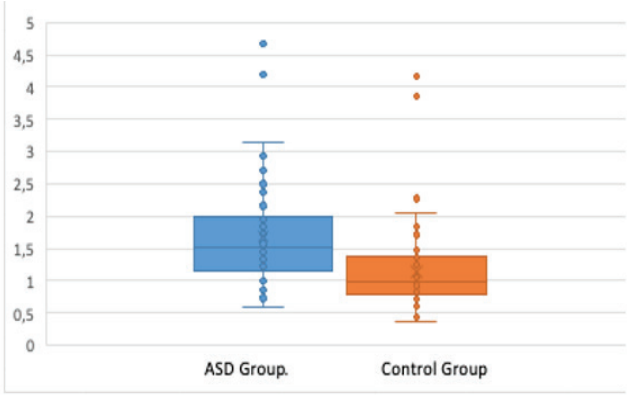

Figure 2. NLR values between ASD patients and the control group

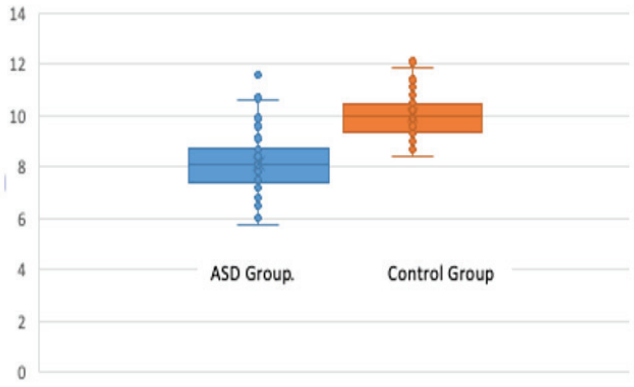

Figure 3. MPV levels between ASD patients and the control group

DISCUSSION

In this study, we aimed to investigate the relationship between ASD and inflammation. For this purpose, CBC was utilized, which is a cheap and practical tool, as well as an indicator of inflammation in recent years (9). Our results support the presence of neuroinflammation in etiopathogenesis of ASD. Importantly, our research showed that NLR values were significantly higher and MPV levels were significantly lower in ASD patients compared to the control group.

According to a growing number of recent studies, a strong inflammatory state is involved with ASD etiopathogenesis (4,5). This inflammatory state is often associated with functional disorders in the immune system (10). Inflammatory activity has been shown in children with ASD using pro-inflammatory biomarker analysis. IL-1β, IL-6, IL-17, IL-12p40, IL-12p70, TNF, LincRNA (THRIL), and S100B are among the biomarkers being linked to ASD (11). However, when assessing inflammation in ASD patients, the most suitable biomarkers are those that are easily investigated, offering cheaper tests that can be performed routinely (12). This is because early diagnosis of ASD is of utmost importance (9). Due to the fact that connections between the neurons of children with ASD are fewer in number and lower in structural quality, regular stimulation of such children is crucial for development of the brain and nervous system, especially during early childhood. As such, the search for simple biomarkers suitable for routine use for early diagnosis of ASD is still ongoing (5).

Studies investigating CBC parameters in ASD patients are limited. In 2013, Sweeten et al. identified only an increase in monocyte counts in ASD in their research involving 31 children with ASD (13). Kutlu et al. conducted a similar study with 64 children with ASD in 2018 and reported only a significant increase in NLR values (14). Additionally, Hesapçıoğlu et al. applied the Childhood Autism Rating Scale (CARS) to and evaluated CBC parameters in 45 ASD patients in 2017, and showed significantly higher NLR values, as well as a positive correlation between autism severity and NLR (12). In contrast, in a study involving 66 children with ASD, Şahin found significantly lower values of NLR in ASD, and failed to identify any correlation between CARS and NLR (15). The relationship between ASD and CBC parameters is still unclear according to the literature (12). Here, we have shown significantly higher NLR values in ASD patients compared to the control group. Importantly, no cut-off values have been determined for NLR in previous research, and our study is novel in this regard. Children with NLR higher than the cut-off value of 2.32 that we report here may be further examined for ASD. Moreover, neutrophil counts are significantly higher in ASD patients than in healthy children. These findings support the presence of a link between inflammation and ASD etiopathogenesis (16).

MPV is a CBC parameter indicating platelet activation in prothrombotic and proinflammatory processes (17). Several neuropsychiatric disorders have been associated with MPV (17-19). In the four studies cited above, no significant relationship have been found between ASD and MPV (12-15). In contrast, here we report significantly lower levels of MPV in ASD patients compared to the control group. Children with MPV levels lower than our cut-off value of 11.75 fL may be considered for further examination for ASD. In order to gather sufficient evidence for this, further research should be conducted with larger samples and prospective study setups.

There are a number of studies in the literature that has identified higher levels of MPV in neuroinflammatory disorders. (17,19-21). This may be explained by the large size of the fresh platelets released from the bone marrow due to inflammation (20). Nonetheless, there are studies suggesting that cytokines released in patients exhibiting advanced inflammatory response reduce the size of platelets, leading to lower levels of MPV. Large platelets are more reactive; since their granule content is denser compared to small platelets, they produce more cytokines and thromboxane A2, which are increasingly required in the acute phase of inflammation. Thus, small platelets are mostly associated with chronic inflammation. The lower levels of MPV found in our study are in agreement to these findings (12,20,21).

The limitations of this work are that it was a retrospective study and that the number of patients was low. Diseases that could affect hemogram results were identified, because it is a retrospective study. Further research should be conducted to investigate the relationship between inflammatory states and ASD severity. Also, in order to assess the response to treatment, studies involving larger sample sizes should be planned.

CONCLUSION

In conclusion, NLR and MPV values may prove to be important biomarkers for ASD, as well as support the presence of neuroinflammation in the etiopathogenesis of ASD. Increased NLR and lowered MPV levels, which can be measured by simple and readily-available laboratory tests, may be used as screening tools and early intervention predictors for ASD. Further research with a larger sample

size may clarify the involvement of inflammation in ASD.

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