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.
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>
<thead>
<tr>
<th>CBC parameters of ASD patients and the control group</th>
<th>ASD Patients (n=67)</th>
<th>Control Group (n=67)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.78 ± 3.60</td>
<td>7.90 ± 2.70</td>
<td>0.813</td>
</tr>
<tr>
<td>Neutrophil (103/mm3)</td>
<td>4.95 ± 2.49</td>
<td>3.86 ± 2.76</td>
<td>0.008</td>
</tr>
<tr>
<td>Lymphocyte (103/mm3)</td>
<td>3.26 ± 1.30</td>
<td>3.10 ± 1.02</td>
<td>0.213</td>
</tr>
<tr>
<td>NLR</td>
<td>1.69 ± 0.81</td>
<td>1.13± 0.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>PLR</td>
<td>112.76 ± 45</td>
<td>115.64 ± 40.45</td>
<td>0.342</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>8.13 ± 1.07</td>
<td>10.01 ± 0.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>Platelet (103/mm3)</td>
<td>340.56 ± 105.48</td>
<td>331.46 ± 87.03</td>
<td>0.591</td>
</tr>
</tbody>
</table>

Figure 1. ROC analysis of NLR and MPV values

Figure 2. NLR values between ASD patients and the control group
66 children with ASD, Şahin found significantly lower severity and NLR (12). In contrast, in a study involving values, as well as a positive correlation between autism patients in 2017, and showed significantly higher NLR Scale (CARS) to and evaluated CBC parameters in 45 ASD Hesapçıoğlu et al. applied the Childhood Autism Rating a significant increase in NLR values (14). Additionally, in a study with 64 children with ASD in 2018 and reported only children with ASD (13). Kutlu et al. conducted a similar investigation, 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 sizes should be planned.
size may clarify the involvement of inflammation in ASD.

Competing interests: The authors declare that they have no competing interest.

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