

**RESEARCH  
ARTICLE**

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## Examination of Systemic Inflammation Related Hemogram Biomarkers in Children and Adolescents with Generalized Anxiety Disorder

### ABSTRACT

**Objective:** In this study, we aimed to examine the complete blood count parameters and blood-based systemic inflammatory markers in children with generalized anxiety disorder (GAD).

**Methods:** Retrospectively, complete blood count of 48 GAD diagnosed children and adolescents and age-gender matched 46 healthy controls were compared in terms of hemoglobin (Hb), erythrocyte distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), white blood cell count (WBC), neutrophil, lymphocyte and monocyte counts and neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and platelet to lymphocyte ratios (PLR). Chi-square test, independent samples t-test and Mann-Whitney U test were used for statistical evaluation.

**Results:** MPV levels were found to be significantly higher ( $p=0.020$ ), while PLT and PDW levels were found to be significantly lower ( $p=0.018$  and  $p=0.011$ , respectively) in children and adolescents with GAD. There was no statistically significant difference in terms of Hb, RDW, PCT, WBC neutrophil, lymphocyte and monocyte counts and NLR, MLR, PLR between case and control groups.

**Conclusions:** Platelet parameters that have been postulated to be associated with inflammation, such as MPV and PDW may be related to possible inflammatory background of GAD in children and adolescents and comprehensive prospective studies are required on this subject.

**Keywords:** Anxiety Disorder, Inflammation, Hemogram, Complete Blood Count, Children and Adolescents

## Yaygın Anksiyete Bozukluğu Olan Çocuk Ve Ergenlerde Sistemik İnflamasyon İlişkili Hemogram Biyobelirteçlerinin İncelenmesi

### ÖZET

**Amaç:** Bu çalışmada yaygın anksiyete bozukluğu olan (YAB) çocuk ve ergenlerde tam kan sayımından bakılan sistemik inflamasyon biyo-belirteçlerinin düzeylerinin incelenmesi amaçlanmıştır.

**Gereç ve Yöntem:** Retrospektif olarak 48 YAB tanılı çocuk ergen ile yaş-cinsiyet açısından eşleştirilmiş 46 sağlıklı kontrol çocuk ve ergenin tam kan sayımı sonuçları; hemoglobin (Hb), eritrosit dağılım genişliği (RDW), trombosit sayısı (PLT), ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW), plateletcrit (PCT), beyaz kan hücre sayımı (WBC), nötrofil, lenfosit ve monosit sayıları ve nötrofil / lenfosit oranı (NLR), monosit / lenfosit oranı (MLR), trombosit / lenfosit oranları (PLR) açısından karşılaştırıldı. İstatistiksel değerlendirmede ki-kare testi, bağımsız örneklem t-testi ve Mann-Whitney U testi kullanıldı.

**Bulgular:** YAB olan çocuk ve ergenlerde, MPV düzeyleri anlamlı olarak yüksek ( $p = 0,020$ ) ve PLT ve PDW düzeyleri anlamlı olarak düşük (sırasıyla  $p = 0,018$  ve  $p = 0,011$ ) bulundu. Hb, RDW, PCT, WBC, nötrofil, lenfosit ve monosit sayıları, NLR, MLR, PLR açısından olgu ve kontrol grupları arasında istatistiksel olarak anlamlı bir farklılık saptanmadı.

**Sonuç:** MPV ve PDW gibi inflamasyonla ilişkili olduğu öne sürülen trombosit parametreleri, çocuk ve ergenlerde YAB'nin olası inflamatuvar arka planıyla ilişkili olabilir ve bu konuda kapsamlı ileriye dönük çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** Anksiyete Bozukluğu, İnflamasyon, Hemogram, Tam Kan Sayımı, Çocuk ve Ergenler

## INTRODUCTION

Anxiety Disorders are considered among the most common psychiatric conditions in children and adolescents with a prevalence of 18 % worldwide (1) and a prevalence of 16.7% in Turkey (2). Anxiety disorders are related to significant impairments in emotional, social, and academic functioning (3) and predispose to other types of psychopathology, in particular depression (4).

Increasing evidence support that immunological and inflammatory processes play an important role on major psychiatric disorders (5,6,7). Inflammatory processes in anxiety disorder has not been examined widely (5). However, there is evidence of increased inflammatory activity in anxiety related disorders (6) and in particular generalized anxiety disorder (8).

When compared to other biomarkers of inflammation, systemic inflammation biomarkers obtained from hemogram are known to be superior for low price, routine use and high reproducibility across laboratories. Furthermore, inflammatory rates are supposed to combine information on both innate and adaptive parts of immunity and to represent a reliable and practical measure of inflammation (9).

We encountered no study evaluating systemic inflammation biomarkers in children and adolescents with anxiety disorder. We encountered only one study investigating the effect of comorbid anxiety disorder on systemic inflammation biomarkers in adolescents with obsessive compulsive disorder (OCD) (10).

In this study, we aimed to evaluate systemic inflammation biomarkers in children and adolescents with generalized anxiety disorder (GAD).

## MATERIAL AND METHODS

Children and adolescents aged between 7–18 years who referred to child and adolescent psychiatry clinic of Düzce University Medical Faculty between July 2017 and February 2019, diagnosed generalized anxiety disorder and whose hemogram results were available, were included in the study. In this retrospective study, we compared blood count of 48 GAD diagnosed children and adolescents with age and gender matched 46 healthy controls. The diagnosis was made according to DSM-5 by an experienced child and adolescent psychiatrist at Düzce University Child and Adolescent Psychiatry Department. Children and adolescents with other psychiatric comorbidities, taking medication, having an infectious or inflammatory disease, chronic medical disease were not included. Patients and controls whose data were missing or incomplete were also not included in the study.

Hemogram parameters, measured from peripheral blood obtained at the initial presentation, were evaluated. Hemoglobin (Hb), erythrocyte

distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), white blood cell count (WBC), neutrophil, lymphocyte and monocyte counts and neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and platelet to lymphocyte ratios (PLR) were recorded. NLR was calculated by dividing the neutrophil count by the lymphocyte count, MLR was calculated by dividing the monocyte count by the and lymphocyte count, PLR was calculated by dividing PLT by lymphocyte count.

Ethics committee approval was obtained from Düzce University Medical Faculty Research Ethics Committee, with approval date 04.03.2019 and protocol number 2019/47.

**Statistical Analyses:** SPSS version 21 (SPSS™, IBM Inc., Armonk, NY) was used for the statistical analyses. Relationships between dichotomous variables were assessed with Pearson chi-square test. Shapiro-Wilk test was used to determine the conformity of the data to normal distribution. Values conforming to normal distribution were presented as mean  $\pm$  standard deviation (SD), values not conforming to normal distribution were presented as median and interquartile range. Variables normally distributed were compared using Student's t-test, and Mann-Whitney U test was used when normal distribution was not established. A p value of 0.05 (two-tailed) was considered significant.

## RESULTS

The mean age of child and adolescents was (151,00  $\pm$  37,93 month) in the case group and (155,74  $\pm$  22,41 month) in the control group. There were 19 (%39,6) male, 29 (%60,4) female children and adolescents in the case and 17 (%36,96) male, 29 (%63,04) female children and adolescents in the control group. The mean age and gender distribution were not significantly different between two groups ( $z = -0.434$ ,  $p = 0.664$  and  $\chi^2 = 0.069$ ,  $p = 0.793$  respectively).

There was no statistically significant difference in terms of Hemoglobin (Hb), erythrocyte distribution width (RDW), plateletcrit (PCT), white blood cell count (WBC), neutrophil, lymphocyte and monocyte counts and neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR) platelet to lymphocyte ratio (PLR) between case and control groups (Table 1).

MPV levels were found to be significantly higher ( $z = -2.320$ ,  $p = 0.020$ ), while PLT and PDW levels were found to be significantly lower ( $t = 2.405$ ,  $p = 0.018$  and  $z = -2.531$ ,  $p = 0.011$ , respectively) in the patient group (Table 2).

**Table1.** Comparison of hemogram parameters of patient and control groups except platelet parameters

	ANX (n=48)	Control (n=46)	P	z/t
<b>Hb</b>	12.94 ± 1.18	13.31 ± 1.53	0.182	-1.346 <sup>a</sup>
<b>RDW</b>	13.70 (13.20-14.98)	13.90 (13.35- 15.13)	0.859	-0.178
<b>WBC</b>	7.64 ± 1.62	7.39 ± 1.46	0.431	0.790 <sup>a</sup>
<b>Lymphocyte</b>	2.44 (2.06-2.80)	2.37 (2.08-3.04)	0.961	-0.049
<b>Neutrophil</b>	1.60 (1.17-2.29)	1.50 (1.20-2.38)	0.414	-0.817
<b>Monocyte</b>	0.55 (0.46-0.64)	0.51 (0.44-0.60)	0.552	-0.594
<b>NLR</b>	1.60 (1.17-2.29)	1.50 (1.20-2.38)	0.540	-0.613
<b>PLR</b>	120.13 (96.34-145.79)	117.41 (87.81-140.54)	0.356	-0.923
<b>MLR</b>	0.22 (0.18-0.29)	0.21 (0.17-0.25)	0.452	-0.753

Mann Whitney U test, <sup>a</sup> student t test**Hb:** Hemoglobin, **RDW:** Erythrocyte distribution width, **WBC:** White blood cell count, **NLR:** Neutrophil lymphocyte ratio, **PLR:** Platelet lymphocyte ratio**Table 2.** Comparison of platelet parameters of patient and control groups

	ANX (n=48)	Control (n= 46)	P	z/t
<b>PLT</b>	315.58 ± 67.34	283.37 ± 62.31	0.018*	2.405 <sup>a</sup>
<b>MPV</b>	8.50 (7.90-9.20)	8.00 (7.40-8.95)	0.020*	-2.320
<b>PDW</b>	16.20 (15.93-16.58 )	16.50 (16.20-16.80)	0.011*	-2.531
<b>PCT</b>	0.24 (0.21-0.27)	0.22 (0.20-0.25)	0.085	-1.721

Mann Whitney U test, <sup>a</sup> student t test, \*p <0.05**PLT:** Platelet count, **MPV:** Mean platelet volume, **PDW:** Platelet distribution width, **PCT:** Plateletcrit

## DISCUSSION

In this study, we examined systemic inflammation biomarkers obtained from hemogram in children and adolescents with GAD and found increased MPV and decreased PLT and PDW levels in children and adolescents with GAD compared to healthy controls. Furthermore, we found no significant alterations in the other hemogram parameters related to systemic inflammation.

There are limited number of studies indicating alterations of inflammatory biomarkers in children and adolescents with anxiety (10,11). We encountered only one study investigating systemic inflammation biomarkers obtained from hemogram in OCD and anxiety comorbidity in children and adolescents. In that study conducted in adolescents, it was reported that a comorbid anxiety disorder in addition to OCD increased the inflammatory response and that adolescents with comorbid anxiety and OCD had significantly higher WBC, neutrophil counts and log neutrophil-lymphocyte ratio than adolescents with pure OCD (10). However, we found no significant difference in terms of white blood cell count (WBC), neutrophil, lymphocyte, monocyte counts or neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), platelet to lymphocyte ratios (PLR) between case and control groups.

We encountered studies investigating only platelet parameters as systemic inflammation biomarkers obtained from hemogram in adult panic disorder (PD) (12-18). The results of those studies are conflicting. Consistent with our result, majority of these studies found increased MPV in the PD group (12-15). In one of those studies anxiety symptoms was found to be positively correlated with MPV (15). The other studies found decreased

MPV in the PD group (16-18). MPV values were also evaluated in schizophrenia, bipolar disorder and depression in adults (19-24). In adolescents, Ozyurt et al studied MPV, PLT and PLR as platelet indices in depression and found no alterations compared to healthy controls (25).

MPV is known as a marker and determinant of platelet action (16). In addition, PDW is considered among the indicators of platelet activity (26). Platelet parameters are considered to reflect the central serotonergic functions and to present windows of brain serotonergic functions (27). On the other hand, sympathetic system activation and serotonergic system dysfunction are considered to play a crucial role in the neurobiology of GAD (28,29).

Stressful life events and anxiety have been indicated to elevate blood catecholamines. Sympatho-adrenal activation is considered to activate platelets through  $\alpha$ -2 receptors, resulting in an increase in platelet volume and activity, and by causing an increase in platelet activity, increased catecholamine levels are considered to activate thrombotic process (12, 30, 31). Increased stress and anxiety have been indicated to cause serotonin to bind 5-HT-2 receptors on platelets and to mediate the release of factors promoting platelet aggregation (32). Supporting this serotonergic hypothesis, treatment with selective serotonin reuptake inhibitors was also shown to cause a decrease in platelet activity (23,33).

The result of increased MPV levels in our study is consistent with the hypothesis of increased platelet activation due to sympathetic system activation and serotonergic imbalance. Also PLT levels were found to be significantly lower in GAD

patients in our study. This finding is consistent with the known inverse relation between MPV and PLT (34). In general, MPV and PDW are also known to be inversely related to each other (35,36). In line with this, decreased PDW levels were found in our study.

Ataoglu et al (23) and Canan et al. (24) showed that MPV values increased in adult major depression. Ataoglu et al additionally showed that MPV values decreased after 8 weeks of escitalopram treatment (23). On the other hand, increased MPV levels are indicated to be related to cardiovascular disorders in adults (37). Among multiple mechanisms, changes in platelet reactivity are considered to be one of the major mechanisms linking depression and cardiovascular disease (38). Interestingly, anxiety symptoms in patients with coronary artery disease have been found to be more effective in increased platelet activity than depression (39). This result may point out the

inflammatory common aspect of depression, anxiety and cardiovascular disease given that increased platelet activity is known to be related to inflammation (40).

This study has some limitations, including relatively small sample size and retrospective design. However, evaluating almost all of the possible systemic inflammation biomarkers obtained from hemogram is the strength of our study.

In conclusion, we suggest that platelet parameters that have been indicated to be associated with inflammation, such as MPV and PDW, may be related to possible inflammatory background of GAD in children and adolescents and comprehensive prospective studies are required on this subject.

#### Disclosure

The authors have no conflict of interest.

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