



Evaluation of Platelet Parameters in Children with Primary Epstein-Barr Virus Infection

Primer Epstein-Barr Virüs Enfeksiyonlarında Trombosit Göstergelerinin Değerlendirilmesi

Platelet Parameters in Epstein-Barr Virus Infection

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Özet

Amaç: Epstein-Barr virüs (EBV) herpes virüs ailesindedir. Çocuklarda yaşam boyu süren enfeksiyonlara ve birkaç kanser tipinin gelişmesinde rol oynamaktadır. Primer enfeksiyonu asemptomatik veya nonspesifik hafif bulgularla seyreder. Bu çalışmamızdaki amacımız primer EBV enfeksiyonlu hastalarda inflamatuvar sürecin trombosit parametrelerini, trombosit lenfosit oranını (TLO) ve nötrofil lenfosit oranını (NLO) etkileyip etkilemediğinin ortaya konulmasıdır. **Gereç ve Yöntem:** Çalışmamız retrospektif bir çalışma olup çalışmamıza 44 primer EBV enfeksiyonlu hasta ile 66 sağlıklı hasta dahil edildi. Her iki grubun demografik verileri ile laboratuvar bulguları kayıt edildi. Verilerin istatistiksel analizinde SPSS 22.0 programı kullanıldı. Niteliksel ve niceliksel verilerin analizinde Student's t-testi, Mann-Whitney U testi, ki kare testleri kullanıldı. İstatistiksel olarak anlamlılık için $p < 0,05$ değeri alındı. **Bulgular:** Ortalama trombosit sayısı, ortalama trombosit hacmi, albümin düzeyleri ve trombosit lenfosit oranı çalışma grubunda kontrol grubuna kıyasla anlamlı düzeyde düşük saptandı (p değerleri sırasıyla 0.028, 0.002, 0.012 ve <0.001 idi). Ortalama ürik asit düzeyi ile trombosit dağılım genişliği düzeyi ise çalışma grubunda kontrol grubuna göre istatistiksel olarak anlamlı düzeyde yüksek saptandı (p değerleri sırasıyla 0.007 ve <0.001 idi). Trombosit dağılım genişliği için 13,7 optimal cutoff düzeyi olarak alındığında [eğri altında kalan alan: 0,774] olup bu değer primer EBV enfeksiyonu için % 81 özgüllüğe, % 79,5 duyarlılığa sahipti ($p < 0.001$). **Tartışma:** Çalışmamız primer EBV enfeksiyonunun trombosit sayısı ve trombosit göstergelerini etkilediğini göstermesi açısından oldukça anlamlıdır. Trombosit parametrelerinin etkilendiği enfeksiyonlarda EBV, ilk düşünülmesi gereken viral etkenlerden biri olmalıdır. Trombosit göstergelerinin EBV enfeksiyonunda oynadığı rolü açıklayacak prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

Epstein-Barr Virüs; Çocuk; Trombosit; Ortalama Trombosit Hacmi; Trombosit Dağılım Genişliği

Abstract

Aim: Epstein-Barr virus (EBV) is a subgroup of the human herpesvirus family that causes lifelong infection and several types of cancer. Primary infection of EBV is asymptomatic or associated with nonspecific, mild symptoms in children. The aim of this study was to evaluate whether the platelet parameters, platelet-to-lymphocyte ratio (PLR), and neutrophil-to-lymphocyte ratio (NLR) were affected by the inflammatory process in children with primary EBV infection. **Material and Method:** Forty-four patients with primary EBV infection and 66 healthy patients were included in our study. Demographic data and laboratory parameters of participants were recorded. Statistical analyses were performed using the SPSS 22.0 software program. Student's t-test, Mann-Whitney U test, and Chi-Square tests were used for evaluation of the qualitative and quantitative values. The values of $p < 0.05$ were considered statistically significant. **Results:** The mean platelet count, mean platelet volume (MPV) level, albumin level, and PLR in the study group were statistically significantly lower than in the control group (p values 0.028, 0.002, 0.012, and <0.001 , respectively). The mean uric acid level and platelet distribution width (PDW) levels in the study group were statistically significantly higher than in the control group (p values 0.007, and <0.001 , respectively). **Discussion:** Our study is important in terms of showing that platelet count and platelet parameters are impacted by primary EBV infections. EBV should be one of the first conditions considered in infections in which platelet parameters are affected. Future prospective studies are needed to explain the role of platelet parameters in EBV infections.

Keywords

Epstein-Barr Virus; Platelet; Mean Platelet Volume; Platelet Distribution Width

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Introduction

Epstein-Barr virus (EBV) is a subgroup of the human herpesvirus family that causes lifelong infection and several types of cancer [1, 2]. Primary infection of EBV is asymptomatic or associated with nonspecific, mild symptoms in children. EBV is the cause of acute infectious mononucleosis in children, especially in adolescents and young people [3]. EBV is mostly transmitted from person to person via oral secretions. There is no effective treatment for EBV or related diseases [4].

Fatigue, fever, tonsillitis, and cervical lymphadenopathy are the most common clinical findings of primary EBV infections. Most patients with primary EBV infections improve without permanent consequences. EBV infections can lead to hematologic symptoms such as thrombocytopenia, neutropenia, hemolytic anemia, and lymphocytosis [5,6].

Platelets have an important role in inflammatory reactions. Various stimuli can induce platelet functions. Platelet-derived secretory molecules are used to measure platelet activity. These molecules include cytokines, chemokines, adhesion proteins, growth factors, and coagulation factors [7,8]. Furthermore, mean platelet volume (MPV) has been reported as a marker of platelet activation. Large platelets are more active and have a tendency to aggregate more than the small ones. MPV levels are determined during routine complete blood cell (CBC) analysis through the use of an automated hematology analyzer. MPV is a convenient evaluation indicator of platelet activation [9].

The inflammatory marker MPV has been investigated in various infectious diseases such as rotavirus gastroenteritis, urinary tract infection, hepatitis B, acute appendicitis, and sepsis. It rises in certain diseases and reduces in others [10-14]. MPV levels generally increase with mild and acute inflammation, whereas they are reduced with severe and chronic inflammation. Large platelets are consumption in the inflammation region [15,16].

Recent studies have demonstrated that some hematological parameters, such as platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR), are indicators of inflammation [17,18].

The aim of this study was to evaluate whether the platelet parameters, PLR, and NLR were affected by the inflammatory process in children with primary EBV infection.

Material and Method

This retrospective study was started after approval by the Institutional Review Board at Adiyaman University, School of Medicine. Our study was carried out in the Department of Pediatrics at the School of Medicine of Adiyaman University in Adiyaman, Turkey. Our study group consisted of 44 patients with primary EBV infection. Sixty-six healthy age- and sex-matched individuals were randomly selected from the well-child pediatric outpatient clinic for the control group of our study.

Demographic data (including age and gender) and laboratory parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, serum albumin (ALB), activated partial thromboplastin time (PTT), prothrombin time (PT), international normalized ratio (INR), hemoglobin (Hb), white blood cell (WBC) count, neutrophil count, lymphocyte count, monocyte count, red blood cell distribution width (RDW), platelet count (PLT), platelet distribution width (PDW), mean platelet volume

(MPV), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) were recorded from each participant's chart.

Exclusion criteria included having diabetes mellitus, asthma, malignancies, or autoimmune diseases and the usage of any drugs that affect platelet function and activity.

Venous blood samples were obtained by venipuncture from the study group and the healthy control group. Primary EBV infection was diagnosed by the presence of IgM antibodies to Viral Capsid Antigen (VCA). Blood samples were taken in tubes with ethylenediaminetetraacetic acid. The Sysmex XT 2000i (Roche Diagnostics GmbH, Mannheim, Germany) automated analyzer device was used for the blood count analysis. MPV levels between 7.0 fL and 11.0 fL were defined as normal.

Statistical analysis

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) version 22 software (SPSS Inc. Chicago, IL, United States). Continuous variables were demonstrated as mean \pm standard deviation (SD). The categorical variables were compared using the χ^2 test. The Kolmogorov-Smirnov test was used to determine whether data were distributed normally or non-normally. Normally distributed data was analyzed using the independent Student t-test. Non-normally distributed data was analyzed using the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cutoff levels of PDW, MPV, and PLR with maximum sensitivity and specificity for primary EBV infection. The correlation between clinical findings and laboratory parameters was determined with the Pearson correlation test. A p level of <0.05 was considered to be statistically significant.

Results

Forty-four patients with primary EBV infection (30 males; 14 females) and 66 healthy patients (44 males; 22 females) were included in our study. Demographic characteristics and laboratory findings of the study group and control group are shown in Table 1. The mean age of the study group was 5.27 ± 3.38 years and the mean age of the control group was 5 ± 3.63 years. There was no statistically significant difference with regard to age and gender between the groups (p values 0.6 and 0.8, respectively). The mean platelet counts, MPV level, albumin level, and PLR in the study group were statistically significantly lower than in the control group (p values 0.028, 0.002, 0.012, and <0.001 , respectively). The mean uric acid level and PDW level in the study group were statistically significantly higher than in the control group (p values 0.007 and <0.001 , respectively). A 64.02 optimal cutoff level of PLR [area under the curve (AUC): 0.722] with a specificity of 68.3% and sensitivity of 70.5% (p

Table 1. Demographic characteristics of the study group and control group

Parameters	Study group (n=44)		Control group (n=66)		p level
	Mean (M) \pm standard deviation (SD)	Mean (M) \pm standard deviation (SD)	Mean (M) \pm standard deviation (SD)	Mean (M) \pm standard deviation (SD)	
Age (years)	5.27 \pm 3.38	5.00 \pm 3.63			0.692
Gender	Male	30	44		0.868+
	Female	14	22		

Student t test or χ^2 test

Table 2. Laboratory findings of the study group and control group

Parameters	Study group (n=44)	Control group (n=66)	p level
	Mean (M) ± standard deviation (SD)	Mean (M) ± standard deviation (SD)	
PDW	16,95±4,14	11,94±3,54	<0.001
MPV	8,18±1,50	8,99±1,19	0,002
PCT	0,24±0,23	0,26±0,07	0,427
Platelet count	252,12±96,50	293,50±95,23	0,028
PLR	53,29±34,95	86,30±46,27	<0.001
NLR	0,83±0,57	1,66±2,04	0,044*
WBC	11,69±4,28	10,42±4,37	0,136
Lymphocyte count	6,24±3,30	4,29±2,30	<0.001
Monocyte count	1,05±0,55	0,94±0,67	0,390
Neutrophil count	3,98±2,18	4,77±3,36	0,541*
ALT	49,50±54,44	15,65±5,19	<0.01*
AST	53,25±28,57	30,95±6,95	<0.01*
LDH	433,82±208,93	376,29±260,97	0,305
Albumin	3,99±0,68	4,47±0,30	0,012
Uric acid	4,46±1,67	3,57±0,84	0,006

Student t test or *Mann Whitney U test

<0.001) was determined in the children with primary EBV infection. A 13.7 optimal cutoff level of PDW (AUC: 0.774) with a specificity of 81% and sensitivity of 79.5% ($p < 0.001$) was determined in the children with primary EBV infection. An 8.64 fL optimal cutoff level of MPV (AUC: 0.686) with a specificity of 63.5% and sensitivity of 70.5% ($P = 0.001$) was determined in the children with primary EBV infection. The ROC curve analysis for PLR and MPV was presented without correction (Figure 1). The MPV levels were negatively correlated with platelet count and PDW levels. The PDW levels were negatively correlated with albumin level. PLR was negatively correlated with titer of VCA IgM antibodies. PLR was positively correlated with NLR (Table 3).

Discussion

This study showed that the PDW levels of children with primary EBV infection were significantly higher than those of the healthy controls. However, MPV levels and PLR of children with

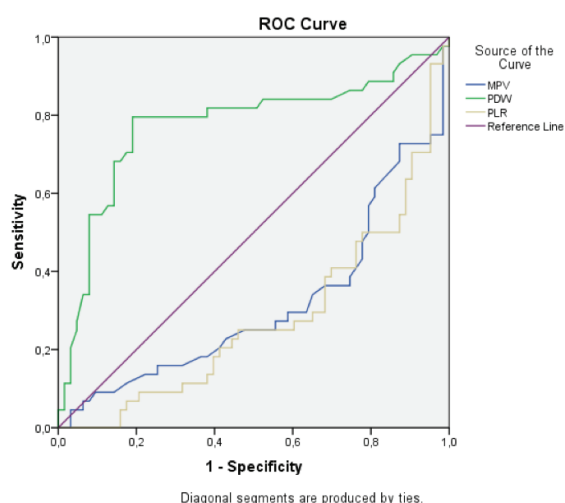


Figure 1. Receiver operating characteristic curve of PDW, platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) for primary EBV infection.

Table 3. Correlation between laboratory parameters

Parameters	Correlation Coefficient (r)	p level
PDW-MPV	0,306-	0,001
PDW-Albumin	0,329-	0,027
PLR-NLR	0,656	0,000
PLR-Monocyte Count	0,279-	0,003
PLR-EBV VCA IgM	0,466-	0,044
Platelet count-MPV	0,219-	0,022

primary EBV infection were significantly lower than those of the healthy controls.

Most of the recent studies have demonstrated that platelet parameters are affected in many diseases and can be used as a marker for different situations [18,19].

Thrombocytopenia is quite frequently seen during the course of most viral infections. EBV infection usually presents with mild decreases in platelet counts and the presence of anti-platelet antibodies usually corresponds to the severity of EBV infections [20]. MPV is a platelet parameter that is a significantly reflective marker for the production rate, function, and activity of the platelets. Larger platelets have higher activity levels than smaller platelets [21,22]. Previous studies have demonstrated that MPV is differently affected in some parasitic [23,24], some bacterial [25-27], and some viral infections [28-30]. MPV was found to be significantly high in some viral infections and low in others.

Ekiz et al. [29] reported that platelet counts were significantly reduced in patients with Crimean-Congo Hemorrhagic Fever (CCHF) and MPV levels were statistically significantly increased. In another study, Turhan et al. [12] showed that MPV levels were significantly higher in the inactive HBsAg carrier. Hu et al. [30] demonstrated that MPV levels were increased in chronic severe hepatitis B and chronic hepatitis B patients. We found lower MPV levels in patients with primary EBV infections compared to the healthy controls in our study. Lower MPV levels in the study group may be related to the acute phase of the EBV infection.

Nkambule et al. [31] found PDW and MPV levels decreased in HIV infections. Tanju et al. [10] demonstrated that MPV levels were significantly lower in rotavirus and MPV was inversely correlated with platelet count. Our study supports the findings that viral infections show decreased MPV levels.

PDW is an indicator of the differences in dimension and dispersion of platelets [32]. PDW is similar to RDW and an index of platelet volume heterogeneity. Normal levels of PDW are between 10% and 17.9%. MPV and PDW are indicators of young platelets in peripheral circulation [33]. A small number of studies have evaluated the effects on PDW levels in some diseases. Cetin et al. [34] showed increased PDW levels in acute myocardial infarction with ST-segment elevation. Akarsu et al. [35] found PDW levels were increased in neonatal sepsis. In contrast, Liang et al. [36] found that PDW levels were significantly decreased in Alzheimer diseases. Topal et al. [37] showed that PDW levels were decreased in atopic eczema. Our study demonstrated increased PDW levels in patients with primary EBV infection.

Lee et al. [19] showed that MPV levels positively correlated with PDW levels and negatively correlated with platelet count in urinary tract infections. We detected a negative correlation between PDW and MPV levels and between platelet count and MPV levels in our study.

Delgado-García et al. [38] found a positive correlation between MPV and albumin in inactive lupus. We determined a negative correlation between albumin and PDW in our study.

PLR and NLR are known as systemic inflammation markers. Many studies have suggested that these markers are related to the prognosis of cardiovascular diseases, sudden deafness, vestibular neuritis, and thrombosis-related diseases [39–41]. Alan et al. [17] found that PLR was significantly higher in patients with Behçet's syndrome. Yazar et al. [42] showed that PLR increased in pregnant women with acute appendicitis. Our study showed that PLR decreased in primary EBV infections.

Our study has several limitations. The first and major limitation of our study is the retrospective and case-control design. The second limitation is the study's small sample population. Prospective and large sample size studies that analyze the alterations of platelet parameters with long-term follow up in EBV infections are needed.

Conclusion

Our study is important in terms of showing that platelet count and platelet parameters are impacted by primary EBV infections. Our study supports the determination that platelet parameters can be used as a marker of inflammation associated with viral infections. EBV should be one of the first conditions to be considered in infections in which platelet parameters are affected. Future prospective studies are needed to explain the role of platelet parameters in the progression and prognosis of EBV infections.

Competing interests

The authors declare that they have no competing interests.

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