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Platelet mass index - a pre-diagnostic tool for infectious mononucleosis

Trombosit kütle indeksi - enfeksiyöz mononükleoz için ön tanı aracı

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ABSTRACT

Objective: As a member of the human herpesvirus family, Epstein-Barr virus (EBV) primarily replicates in lymphocytes but also may replicate in the epithelial cells of the pharynx and parotid duct. The infection is spread primarily by saliva and the incubation period is four to eight weeks. Infectious mononucleosis (IM) is a clinical syndrome caused by EBV that is particularly common in adolescents and children. Typical features of IM include fever, pharyngitis, adenopathy, malaise, and an atypical lymphocytosis. Splenomegaly, hepatomegaly, jaundice, and splenic rupture can occur. The platelet mass index (PMI) is related to platelet functionality and platelet function has an effect on the inflammation cascade. This study is planned to examine the role of PMI in the pre-diagnosis of IM.

Methods: Between the years 2010-2019, 274 patients who were tested for EBV antibodies were included in the study and the PMI values of EBV positive group was compared to EBV negative and the control group.

Results: EBV positive group has lower PMI values than EBV negative and control group.

ÖZET

Amaç: İnsan Herpesvirüs ailesinin bir üyesi olan Epstein-Barr virüs (EBV) başta lenfositler olmak üzere farenks ve parotis kanalının epitel hücrelerinde çoğalmaktadır. Tükürük yoluyla yayılan enfeksiyonun kuluçka süresi dört ile sekiz haftadır. Enfeksiyöz mononükleoz (EM), özellikle ergenlerde ve çocuklarda yaygın olan EBV'nin neden olduğu bir hastalıktır. EM'nin tipik özellikleri arasında ateş, farenjit, adenopati, halsizlik ve atipik lenfositoz bulunmakta ve splenomegali, hepatomegali, sarılık ve dalak rüptürü meydana gelebilmektedir. Trombosit kütle indeksi (TKİ), trombosit işlevselliği ile ilişkilidir ve trombositlerin inflamasyon kaskadına etkisi konusunda bilgi verebilmektedir. Bu çalışma, EM ön tanısında TKİ'nin rolünü incelemek amacıyla planlanmıştır.

Yöntem: Çalışmaya 2010-2019 yılları arasında EBV antikor testi yapılan 274 hasta dahil edilmiştir. EBV antikoru pozitif grubun TKİ değerleri EBV antikoru negatif olan grup ve kontrol grubu ile karşılaştırılmıştır.

Bulgular: EBV pozitif grubun, EBV negatif ve kontrol grubuna göre daha düşük TKİ değerlerine sahip olduğu saptanmıştır.

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Conclusion: EPMI can be easily calculated using a CBC test and can be a used as a pre-diagnostic tool for the existence of IM.

Key Words: Epstein-Barr virus, infectious mononucleosis, platelet mass index

Sonuç: Tam kan sayımı değerlendirilirken TKİ kolayca hesaplanabilmektedir. Elde edilen veriler klinik olarak EM şüphesi olduğunda ön tanı aracı olarak TKİ'nin yol gösterici olabileceğini düşündürmektedir.

Anahtar Kelimeler: Epstein-Barr virüs, enfeksiyöz mononükleoz, trombosit kütle indeksi

INTRODUCTION

In developing countries and in socioeconomically disadvantaged populations of industrialized countries, primary Epstein-Barr virus (EBV) infection usually occurs during infancy and early childhood and is generally asymptomatic or only mildly symptomatic. In more affluent populations in industrialized countries, infection during early childhood is still more common but about one-third of cases of infection occur during adolescence and early adulthood (1).

EBV, a member of the human herpesvirus family, is a linear, double-stranded DNA virus that was initially isolated from a cultured Burkitt lymphoma cell line by Epstein et al in 1964. EBV primarily replicates in lymphocytes but also may replicate in the epithelial cells of the pharynx and parotid duct. The infection is spread primarily by saliva (oral transmission), and the incubation period is four to eight weeks (2, 3).

Infectious mononucleosis (IM) is a clinical syndrome caused by EBV that is particularly common in adolescents and children. The vast majority of persons with IM, especially children, recover uneventfully without any complications, although a wide range of clinical complications of acute IM has been reported (1). Symptomatic treatment, the mainstay of care, includes adequate hydration, analgesics, antipyretics, and adequate rest (3).

Platelets are non-nucleated cells in blood that are produced in bone marrow and derived from megakaryocytes. Besides stopping bleeding and achieving hemostasis, they play a role in inflammation due to the activation of cytokines. Inflammation changes the structure of platelets and causes alterations in mean platelet volume (MPV) (4).

The platelet mass index (PMI) is related to platelet functionality; a new concept that is used in the Neonatal Intensive Care Unit. Nowadays, in order to reduce unnecessary transfusions, utilizing the PMI is investigated. In very-low-birth-weight infants, those infants with severe bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP) were more likely to have lower PMI compared to those without these morbidities. In addition, infants with early onset neonatal sepsis (EOS), late onset neonatal sepsis (LOS) and respiratory distress syndrome and infants who required surfactant and mechanical ventilation had a decrease in PMI (4-6).

Considering that low PMI indicates platelet function, and platelet function affects the inflammation cascade, we planned to examine the role of PMI in the pre-diagnosis of IM.

MATERIAL and METHOD

The study was performed at the TOBB Economy and Technology University, Faculty of Medicine Department of Paediatrics. In the age range of 0-15 years, 274 patients were investigated retrospectively. Complete blood counts (CBC) and EBV antibodies used for the diagnosis of IM of these patients were analysed

on the day they were admitted to the hospital.

Blood samples were collected into 2-mL (K2) EDTA vacutainer tubes (Becton Dickinson, USA) for CBC and into 8,5-mL SST™ II Advance vacutainer tubes (Becton Dickinson, USA) for serological analysis. All whole samples were kept at room temperature (18-25°C) until testing and were processed within 30 minutes from venepuncture. Sera were separated after centrifugation at 4500 rpm for 10 minutes, stored at 2-8°C and analysed for EBV antibodies on the same day.

CBC was analysed using Sysmex XN-1000 (Sysmex Co., Japan), C-reactive protein (CRP) and ferritin levels using Cobas 6000 (Roche Diagnostics Co., Mannheim, Germany) and EBV IgM (Anti-VCA (Viral capsid antigen) GP 125 IgM, anti-VCA P19 IgM, anti EBNA-1 (EBV Nuclear Antigen) IgM, anti P22 IgM and anti-EA-D (Early Antijen) IgM antibodies) using Euroline Anti-EBV Profile 2-IgM (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany)

Patients were diagnosed as IM if they were positive for EBV Anti-VCA G IgM and anti-EA IgM antibodies. 44 patients out of 274 were diagnosed as IM regarding to this condition. Control group was formed from 41 patients between the ages 1 and 15 who were admitted to same department. We defined Group 1 (n=44) as EBV positive, group 2 as EBV negative (n=230) and Group 3 (n=41) as control.

In order to exclude anemia or infection, ferritin value above 20 ng/ml (normal range: 7,0-140 ng/ml), leukocyte count under 10 x 10^3/µl (normal range: 4,5-11,0 x 10^3/µl) and CRP level under 5 mg/L (normal range : 0-5,0 mg/L) were taken as inclusion criteria for the control group. All CBC parameters were within the reference ranges and there was no transfusion history within the previous 30 days.

PMI values were calculated by analysing the blood counts of both groups using the formula: PMI = mean platelet volume (MPV) x Platelet count/1000. Mean, median and standard deviation values (SD) are calculated individually for the groups. The results of internal quality control (XN Check) and external quality control (College of American Pathologist, Hematology Auto Differentials) results were given in Table 1.

Table 1. The results of internal and external quality control

			Mean	SD	CV*
Internal Quality Control	Platelet count (x10^3/µL)	Level 1	85	4,0	4,8
		Level 2	256	9,0	3,5
		Level 3	607	10,4	1,7
	MPV (fL)	Level 1	8,5	0,25	2,9
		Level 2	9,2	0,13	1,4
		Level 3	8,9	0,07	0,8
External Quality Control	Platelet count (x10^3/µL	Level 1	512,9	11,5	2,2
		Level 2	1403	6,1	0,4
		Level 3	337,5	7,8	2,3
		Level 4	228,1	6,3	2,8
		Level 5	54,6	2,7	4,9
	MPV (fL)	Level 1	9,44	0,13	1,4
		Level 2	9,58	0,26	2,7
		Level 3	9,71	0,12	1,2
		Level 4	9,65	0,17	1,8
		Level 5	10,06	0,32	3,2

** CV: coefficient of variation*

Statistical analyses were performed using commercially available statistical software package (SPSS, version 18, Chicago, IL, USA). For the statistical comparison of the variables between the control and study groups (group 1, group 2 and group 3) ANOVA test was used. *p* values < 0.05 were considered to indicate statistical significance.

The study was approved by the TOBB Economy and Technology University, Faculty of Medicine Clinical Research Ethics Committee (Date: 18.01.2023 and Number: KAEK-118/149).

RESULTS

UPMI values of group 1 and 2 (Table 2), group 1 and 3 (Table 3), and group 2 and 3 (Table 4) were compared to see the difference between the groups. A statistically significant difference was found between EBV positive and negative group and EBV positive and control group (p<0,05).

Table 2. Group 1 and group 2 PMI mean, SD and P-values

	Group 1 (EBV Positive) (N=44)	Group 2 (EBV Negative) (N=230)	P-Values
PMI	2340±725	2707±823	**0.013***

**statistically significant (p<0,05)*

Table 3. Group 1 and group 3 PMI mean, SD and P-values

	Group 1 (EBV Positive) (N=44)	Group 3 (Control) (N=41)	P-Values
PMI	2340±725	2742±565	**0.048***

**statistically significant (p<0,05)*

Table 4. Group 2 and group 3 PMI mean, SD and P-values

	Group 2 (EBV Negative) (N=230)	Group 3 (Control) (N=41)	P-Values
PMI	2707±823	2742±565	0.961

DISCUSSION

Infectious mononucleosis is a disease caused by EBV and notably seen in infancy, early childhood and adolescents. Typical features of IM include fever, pharyngitis, adenopathy, malaise, and an atypical lymphocytosis. Splenomegaly, hepatomegaly, jaundice, and splenic rupture can occur in patients with IM, but these complications are rare (3). Up to 98% of all patients with IM have sore throat, lymphadenopathy, fever, fatigue, and tonsillar enlargement. Pharyngeal inflammation (85%) and transient palatal petechiae (50%) are also common. Bilateral posterior cervical lymphadenopathy is typical, but anterior cervical lymphadenopathy is possible (7). The syndrome is characterized by an

absolute and relative lymphocytosis, an increased proportion of atypical lymphocytes and positive heterophile antibody test (3). The antibodies (Anti-VCA, Anti EBNA and anti-EA) to EBV-associated antigens are used for diagnosis of primary and past infection. Additionally, easily accessible laboratory tests can be used for pre-diagnosis of IM.

Akışın et al. investigated the relationship between Platelet-to-lymphocyte ratio (PLR) and IM positivity. They showed that PLR value is lower for IM positive group when the cut-off value is 48. If the cut-off value was taken as 48, sensitivity and specificity were found to be 50% and 95%, respectively. On this basis, they supported that low PLR values can be used as a pre-test for IM (8).

Apart from stopping bleeding and sustaining hemostasis, platelets play incredibly important roles in inflammation, like primary cells leading to cytokine release, such as T-lymphocytes and macrophages. Platelets are affected by this ''cytokine storm'' and have diverse responses. Three different types of granules (α-granules, dense granules and lysosomes) are secreted from platelets when they are activated by cytokines in inflammation. These released chemokines have been shown to play a role in the early immune response as acute phase reactants; function as neutrophils, granulocytes and monocytes; and even have direct antimicrobial effects. It has been reported that in cases of inflammation, depolymerization of the microtubules and alterations in the actin polymerization structure of the platelets lead to change in shape and a decrease in the size of the platelets. This mechanism has been the source for many studies investigating changes in mean platelet volume (MPV) in many diseases (4).

PMI is associated with platelet functionality because larger platelets are enzymatically more active than smaller ones (6). It is also a new parameter that has been investigated in a variety of diseases. Kahvecioglu et al. reported that, in order to prevent unnecessary platelet transfusions in NICU, they utilized PMI values. They found that if transfusion decisions made with the current guideline and due to PMI values, there was an 11.5% reduction in platelet transfusions. They supported considering PMI values for lower transfusion rates (9).

Okur et al.'s study on PMI and inflammation-related morbidities of prematurity including sepsis in very low birth weight infants, indicated that premature infants with these morbidities had lower PMI levels in early postnatal life than infants without these diseases and stated that this relationship could be associated with the role of platelet function (which is related to PMI) in the inflammatory process (4). Ilhan et al. found PMI lower in the severe transient tachypnea of the newborn and indicated that low PMI reflects platelet function which contributes to the initiation of inflammatory cascades (6).

Considering that the internal and external quality control results and coefficient of variation values for platelet count and MPV were within acceptable limits (CVs < 5%), in our study, we found that EBV positive group has lower PMI values than EBV negative and control group (10). Therefore, we can interpret that lower PMI values can be used as a parameter for the pre-diagnosis of IM disease in the paediatric population.

In conclusion; measuring EBV specific antibodies is a complicated, time consuming process and not an easy way to access in small healthcare centers. Besides PMI can be easily calculated using a CBC test and can be a useful pre-diagnostic tool for the existence of IM.

ETHICS COMMITTEE APPROVAL

* The study was approved by the TOBB Economy and Technology University, Faculty of Medicine Clinical Research Ethics Committee (Date: 18.01.2023 and Number: KAEK-118/149).

CONFLICT OF INTEREST

The author declares no conflict of interest.

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