Do Routine Laboratory Parameters Predict the Disease Activity in Children with Behçet's Disease?

Rutin Laboratuvar Parametreleri Behçet Hastalığı olan Çocuklarda Hastalık Aktivitesini Öngörüyor mu?

Deniz Gezgin Yildirim[1], Sevcan A. Bakkaloglu[2]

[1]Department of Pediatric Rheumatology, Faculty of Medicine, Gazi University, Ankara, Turkiye
2Department of Pediatric Rheumatology, Faculty of Medicine, Gazi University, Ankara, Turkiye

ABSTRACT

Objective. Juvenile Behçet's disease (jBD) is a multi-systemic inflammatory disorder characterized by recurrent episodes of oral and genital aphthae, and cutaneous, gastrointestinal, neurological, articular, ocular and vascular manifestations. The main aim of this study was to assess the value of routine laboratory parameters for predicting disease activity in children with jBD.
Methods. The demographic features and laboratory findings, including white blood cell, neutrophil (NEU), lymphocyte (LYM) and platelet (PLT) counts, neutrophil/ lymphocyte ratio (NLR), platelet/ lymphocyte ratio (PLR), mean platelet volume/platelet ratio (MPR) values were retrospectively evaluated between jBD patients with active and inactive, and compared with those in healthy peers. Inactive jBD was accepted as the absence of any clinical symptoms, while active jBD was accepted as presence of at least two clinical symptoms related with jBD.
Results. Thirty six patients with jBD, and 58 sex- and age-matched healthy children were enrolled into this study. Median age at diagnosis of jBD was 13 (min – max: 5-17) years. 12 patients had active and remaining 24 inactive disease at enrollment. Active jBD group had significantly higher mean values for NLR, PLR, and lower MPV compared to inactive patients (p= 0.026, p= 0.039, and p= 0.07, respectively). PLR was revealed as an independent factor for predicting disease activity in jBD patients (p= 0.035, OR [95% CI] = 0.988 [0.978-0.999]).
Conclusion. This study has shown that active jBD patients had increased values of NLR and PLR. Among them PLR was the possible risk predictor for disease activity. These parameters are easily accessible inflammatory markers that may help detecting active disease in the early phase to prevent complications and to guide the therapy.

Keywords: Disease activity, juvenile Behçet's disease, mean platelet volume/platelet ratio, neutrophil/lymphocyte ratio, pediatric rheumatology, platelet/lymphocyte ratio

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ÖZET

Amaç. Juvenil Behçet hastalığı (jBH), tekrarlayan oral ve genital aft atakları ve kutanöz, gastrointestinal, nörolojik, artiküler, oküler ve vasküler bulgularla karakterize multisistemik inflamatuar bir hastalıktır. Bu çalışmanın temel amacı, jBH'li çocuklarda hastalık aktivitesini öngörmek için rutin laboratuvar parametrelerinin değerini değerlendirmektir.
Yöntem. Demografik özellikler ve beyaz kan hücresi, nötrofil (NEU), lenfosit (LYM) ve trombosit (PLT) sayıları, nötrofil/lenfosit oranı (NLR), trombosit/lenfosit oranı (PLR), ortalama trombosit hacmi/trombosit oranı (MPR) değerlerini içeren laboratuvar bulguları, aktif ve inaktif jBH hastaları arasında retrospektif olarak değerlendirildi ve sağlıklı akranlarıyla karşılaştırıldı. İnaktif jBH herhangi bir klinik semptomun yokluğu olarak kabul edilirken, aktif jBH hastalık ile ilişkili en az iki klinik semptomun varlığı olarak kabul edildi.
Bulgular. Otuzaltı jBH hastası ile cinsiyet ve yaş açısından eşleştirilmiş 58 sağlıklı çocuk çalışmaya dahil edilmiştir. JBH tanısı konulduğunda ortanca yaş 13 (min - maks: 5-17) yıldı. Kayıt sırasında 12 hastada aktif, kalan 24 hastada ise inaktif hastalık vardı. Aktif jBH grubu, inaktif hastalara kıyasla NLR, PLR ve düşük MPV için anlamlı olarak daha yüksek ortalama değerlere sahipti (sırasıyla p= 0.026, p= 0.039 ve p= 0.07). PLR, jBH hastalarında hastalık aktivitesini öngörmede bağımsız bir faktör olarak ortaya çıkmıştır (p= 0.035, OR [%95 CI] = 0.988 [0.978-0.999]).
Sonuç. Bu çalışma, aktif jBH hastalarının NLR ve PLR değerlerinde artış olduğunu göstermiştir. Bunlar arasında PLR, hastalık aktivitesi için olası risk belirleyicisi olmuştur. Bu parametreler, komplikasyonları önlemek ve tedaviyi yönlendirmek için aktif hastalığın erken evrede tespit edilmesine yardımcı olabilecek kolay erişilebilir enflamatuar belirteçlerdir.

Anahtar Sözcükler: Hastalık aktivitesi, juvenil Behçet hastalığı, ortalama trombosit hacmi/trombosit oranı, nötrofil/lenfosit oranı, pediatrik romatoloji, trombosit/lenfosit oranı

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ORCID IDs: D.G.Y.0000-0002-4823-2076,S.A.B.0000-0001-6530-9672

Address for Correspondence / Yazışma Adresi: Deniz Gezgin Yıldırım, MD Gazi University Faculty of Medicine Department of Pediatric Rheumatology Ankara, Turkiye E-mail: gezgindeniz@gmail.com

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INTRODUCTION

Behçet's disease (BD) is a multi-systemic inflammatory disorder characterized by recurrent attacks with oral and genital aphthae, and cutaneous, gastrointestinal, neurological, articular, ocular, and vascular manifestations (1). Ancient 'Silk Road', involving East Asia and the Mediterranean Basin, is the most commonly seen places for BD (2). Human leukocyte antigen-B51 (HLA-B51) allele is associated genetic susceptibility factor for BD (3). Clinical findings of BD usually appear at the age of 30–40 but can also be appear in pediatric ages (4). Diagnosis is mainly based on clinical symptoms. Colchicine is the main treatment for the aphthae. Corticosteroids, immunosuppressive and biologic therapies, are used in patients with BD who have uncontrolled recurrent aphthae attacks and an organ involvement associated with the disease (5).

Due to a variable course of BD, predictive laboratory tests for estimating disease activity have been investigated in adult BD patients (6-11). Defining a laboratory parameter as a disease activity predictor may provide early recognition of the severe disease course and alert physicians to follow-up patients more closely. Until now, inexpensive and easily available, routine laboratory parameters including hemoglobin (Hb), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM) and platelet (PLT) counts, mean platelet volume (MPV), MPV/PLT ratio (MPR), NEU/LYM ratio (NLR) and PLT/LYM ratio (PLR) have been investigated in adult BD patients (6-11). Few studies suggested significant differences in NLR and PLR values in patients with BD compared with healthy people (6-8,10,11). However, to our knowledge there is still no data about laboratory parameters predicting disease activity in juvenile BD (jBD).

In this study, we aimed to assess the value of routine laboratory parameters, including WBC, NEU, LYM and PLT, MPV, NLR, PLR, and MPR for predicting disease activity in children with BD.

METHODS

The study included 36 children who were younger than 18 years of age, diagnosed with jBD and followed-up in the Pediatric Rheumatology Department of XXX University Medical Faculty between May 2015 and June 2019. Patients were diagnosed with jBD according to Pediatric BD group (PEDBD) criteria (12). The PEDBD criteria involve six contents; recurrent attacks of oral aphthae, genital ulcers, cutaneous lesions, ocular, neurologic and vascular manifestations, respectively. ≥3 items fulfill the diagnosis of jBD according to PEDBD criteria (12). Fifty-eight sex- and age-matched healthy controls who referred to the outpatient clinic between May 2015 and January 2019 were included as control group. Exclusion criteria were having comorbidities accompanying to jBD and/or concurrent infection at the time of laboratory test evaluation.

The patients' characteristics, including age, gender, age at onset of jBD findings, age at diagnosis, family history for BD, oral apthae, genital aphthae, erythema nodosum, acneiform lesions, vascular, neurologic, and ocular involvement, laboratory findings, including HLA-B51 status, WBC, Hb, NEU, LYM and PLT counts, MPV, NLR, PLR and MPR values, and given treatments were retrospectively recorded from the medical charts. Patients who were followed-up for at least 6 months were included in the study. jBD patients were divided into two groups according to disease activity to compare laboratory parameters: Group 1 comprised active jBD patients and group 2 inactive jBD patients. In addition, group 3 comprised healthy children. Inactive jBD was accepted as the absence of any clinical symptoms, while active jBD was accepted as involvement of at least two clinical symptoms of the followings; oral ulcers, genital ulcers, active uveitis, cutaneous lesions, arthritis and thrombosis-thrombophlebitis, neurological and gastrointestinal involvement.

The complete blood count analyses studied in the same Coulter analyzer for standardize the results. NLR was calculated by spliting the neutrophil and lymphocyte count, while PLR was calculated by spliting PLT and LYM count, and MPR was calculated by spliting MPV and PLT. The present study was approved by the ethics committee of the Gazi University Medical Faculty (15.01.2019, approval number:79), and was carried out in accordance with the Helsinki Declaration.

Statistical Analysis

SPSS software version 23 (IBM Corps., Armonk, NY) was used for the evaluation of the statistical analysis. For continuous variables mean and standard deviation (SD) or median, minimum (min) and maximum (max) were calculated, while for categorical variables number and percentages were given. The Kolmogorov-Smirnov test was performed to assess the normality of the variables. The Chi-square test or Fisher's exact test was performed to compare the categorical variables. The differences between two independent groups were evaluated by Independent Sample t–test for normal distributed variables, and Mann–Whitney U test for non–normal distributed ones. A multiple linear logistic regression test was performed to predict independent predictors for disease activity in jBD patients. A p-value <0.05 was accepted statistically significant.

RESULTS

Baseline characteristics of patients

The demographic characteristics and clinical and laboratory findings of jBD patients are summarized in Table 1. There were 12 active jBD patients, 24 inactive jBD patients and 58 healthy controls in the study. Median age at diagnosis was 12 (range: 5-17) years. The female/male ratio was 17/19. The most frequent clinical finding was recurrent oral ulcers (n=36, 100%), followed by genital aphthae (n=24, 66%), skin manifestations (n=18, 50%), ocular (n=18, 50%), vascular (n=7, 19%) and neurological signs (n=4, 11%). The rate of family history for BD positivity was 33%. HLA-B51 antigen was detected in 19 (53%) patients.

Treatments were performed for each patient according to the severity of aphthae lesions and the presence of organ involvements. All patients used colchicine therapy. Corticosteroid therapy was administered to the patients who were in activation periods related with BD, such as recurrent severe mucosal aphthae, genital ulcers, uveitis and/or other organ involvements. Other immunosuppressive therapies, including azathioprine, cyclosporine, cyclophosphamide, and mycophenolate mofetil, were performed as steroid-sparing agents. Intravenous pulse cyclophosphamide was the most preferred therapy, followed by azathioprine as maintenance therapy, in cases with life-threatening events. Biologic therapies such as anti–tumor necrosis factor therapies (etanercept, adalimumab, and/or infliximab) were prescribed to the patients who had an organ involvement. Low molecular weight heparin in addition to immunosuppressive treatment was prescribed to patients with vascular thrombosis.

Laboratory parameters of jBD patients and healthy controls

The comparison of laboratory tests between active and inactive jBD patients and healthy peers were summarized in Table 2. Active jBD group had significantly higher values of NLR and PLR (p= 0.026 and p= 0.039); and lower MPV value compared to inactive jBD patients (p= 0,007). Compared to active jBD patients and healthy controls, active jBD group had significantly higher mean values for WBC and NEU, in addition to higher values of NLR and PLR (p= 0,011, p= 0,001, p= 0,000, and p= 0,008, respectively).

Evaluation of the possible risk factors for the disease activity

All laboratory parameters were evaluated to predict the disease activity of jBD by a multiple linear logistic regression analysis and presented in Table 3. PLR (p= 0.035, OR:0.988, CI 95%: 0.0978-0.999) was found as an independent factor to predict active and inactive disease in jBD patients.

Table 1. Demographic, clinical and laboratory findings of juvenile Behçet's disease patients.

	n (%)	median (min-max)
Demographic variables		
Total	36 (100%)	
Female/Male	17(47%)/19(53%)	
Age at disease onset (years)		11 (4-17)
Age at diagnosis (years)		12 (5-17)
Family history of BD	12 (33%)	
Clinical Manifestations		
Oral aphthous lesions	36 (100%)	
Genital ulcers	24 (66%)	
Pseudo-folliculitis	13 (36%)	
Erythema nodosum	5 (14%)	
Eye involvement	18 (50%)	
Vascular jBD	7 (19%)	
Neuro-jBD	4 (11%)	
HLA-B51 positivity	19 (53%)	

jBD; juvenile Behçet's disease.

Table 2. The comparison of laboratory tests between active jBD patients, inactive jBD patients and healthy controls.

Variables	Active jBD patients (n=12)	Inactive jBD patients (n=24)	Control group (n=58)	p1 value	p2 value	p3 value
WBC count (x 10^3/uL)	10,02 (4,05-19,6)	8,37 (4,41-27,72)	7,97 (2,83-11,71)	0,204	0,011	0,112
Hb (g/dL)	13,26±1,66 13,29 (9,8-16,6)	13,41±1,64 13,5 (8,5-15,9)	13,75±1,22 13,85 (10,9-16,3)	0,724	0,155	0,271
PLT count (x 10^3/uL)	335,5±75,09 334,5 (184-487)	338,16±102,55 321 (166-637)	325,97±74,20 326 (187-513)	0,913	0,611	0,527
NEU count (x 10^3/uL)	5,81 (2,19-16,63)	4,55 (1,89-23,59)	3,86 (2,14-8,15)	0,101	**0,001**	0,097
LYM count (x 10^3/uL)	2,39 (0,8-4,83)	2,73 (1,2-12,8)	2,74 (1,63-5,14)	0,109	0,052	0,713
MPV (fL)	9,15 (6,7-11,15)	9,6 (6,82-13)	9,7 (7,8-12,7)	**0,007**	0,076	0,746
NLR	2,21 (0,86-9,13)	1,67 (0,22-8,64)	1,38 (0,42-3,67)	**0,026**	**0,000**	0,134
PLR	154,85±68,19 145,72 (55,2-375)	121,22±44,29 113,65 (17,97-226,47)	109,8 (62,09-194,24)	**0,039**	**0,008**	0,635
MPR	0,02 (0,01-0,05)	0,02 (0,01-0,08)	0,02 (0,02-0,06)	0,247	0,067	0,357
CRP (mg/L)	22,1 (1-98)	2,6 (0,1-19)	2 (1-16,13)	**0,000**	**0,000**	**0,013**
ESR (mm/hr)	35 (9-74)	10 (4-103)	12 (2-23)	**0,000**	**0,000**	0,887

jBD; juvenile Behcet's disease; Hb; Hemoglobin, PLT; platelet, WBC; white blood cell, NEU; neutrophil, LYM; lymphocyte, NLR; neutrophil/lymphocyte ratio, PLR; platelet/lymphocyte ratio, MPV; mean platelet volumes, MPR; MPV /platelet count ratio, CRP; C-reactive protein, and ESR; erythrocyte sedimentation rate. p1 exhibits the comparison between active jBD patients and inactive jBD patients; p2 exhibits the comparison between active jBD patients and healthy controls; and p3 exhibits the comparison between inactive jBD patients and healthy controls.

**Data presented as median (minimum-maximum).*

Table 3. Univariate and multivariate logistic regression analysis between active and inactive juvenile Behçet's disease patients.

	Univariate	Multivariate	
	p value	OR (95% CI)	p value
WBC count (x 10^3/uL)	0,295	0,937 (0,827-1,061)	0,303
Hb (g/dL)	0,719	1,060 (0,772-1,454)	0,719
PLT count (x 10^3/uL)	0,911	1,000 (0,995-1,006)	0,911
NEU count (x 10^3/uL)	0,267	0,931 (0,818-1,059)	0,275
LYM count (x 10^3/uL)	0,093	1,441 (0,874-2,376)	0,152
MPV (fL)	0,065	1,439 (0,963-2,150)	0,076
NLR	0,054	0,787 (0,612-1,013)	0,063
PLR	0,020	0,988 (0,978-0,999)	0,035
MPR	0,226	0,686 (0,355-1,258)	0,249
CRP (mg/L)	0,000	0,772 (0,676-0,881)	0,000
ESR (mm/hr)	0,000	0,944 (0,910-0,978)	0,002

WBC; white blood cell, Hb; Hemoglobin, PLT; platelet, NEU; neutrophil, LYM; lymphocyte, NLR; neutrophil/lymphocyte ratio, PLR; platelet/lymphocyte ratio, MPV; mean platelet volumes, MPR; MPV /platelet count ratio, CRP; C-reactive protein, and ESR; erythrocyte sedimentation rate.

DISCUSSION

Behçet's Disease is a chronic autoinflammatory disease with recurrent multi-organ involvement (1). Patients may have inactive and stable course, although new complaints/organ involvements related disease activation may occur during follow-up. In order to predict disease activity, laboratory parameters had been studied in adult patients (6-11). To the best of our knowledge, this is the first study investigating of the predictive value of routine laboratory parameters like leukocyte, neutrophil, lymphocyte and PLT counts, NLR, PLR, MPV and MPR for predict the severity of the disease in children with BD. This study demonstrated that NLR, PLR, and MPV values were useful non-invasive predicting factors in differentiating active and inactive jBD patients. Our results suggested that NLR and PLR were higher, whereas MPV was lower in active jBD patients compared to inactive jBD patients. Furthermore, only PLR was found as a possible risk predictor for active disease in jBD.

WBC count has been using as an inflammation predictor. NLR and PLR have been reported as an indicator of systemic inflammation in various rheumatic conditions (13). Previous studies in adults showed that WBC, NEU, NLR, PLR and PLT levels were higher, whereas LYM and Hb levels were lower in BD disease compared to the healthy subjects (7,9,10,14). In our study, we determined higher values of WBC, NEU, NLR, but no significant differences in Hb, PLT, LYM and PLR values in patients with jBD and healthy controls. Additionally, MPV levels in jBD patients were significantly lower compared to healthy peers. There are conflicting results regarding MPV values in BD including higher (15), lower (16,17) or similar (9) values compared to healthy subjects. Higher MPV levels in BD were associated with thrombosis (15).

Balkarlı et al. evaluated the MPV and NLR values according to disease activity in adult patients with BD, and they described a higher NLR value in active BD group than inactive BD group similar to our results, in contrast they did not find any association with MPV value according to disease activity (6). Rifaioglu et al. reported higher WBC and NLR levels in adult patients with active BD than inactive BD (7). However, WBC value was similar between active and inactive jBD patients in our cohort. Hammad et al. and Jiang et al. found a positive correlation between disease activity and NLR as well as PLR values (8,10). Furthermore, Hammad et al. suggested NLR was superior to PLR as an indicator of disease activity in BD, while Jiang et al. suggested PLR as the best predictor of BD (8,10). According to mild, moderate and severe disease activity, PLR and MPV were significantly different among patients with jBD. In contrast, PLR, NLR and MPV levels were not different in jBD patients with or without organ involvement. Finally, they suggested that NLR is an independently associated factor with BD (9). Additionally, NLR was found as a marker of uveitis in BD (11).

The main limitations of this study are its retrospective design and small sample size, which may limit the value of statistical analysis.

In conclusion, NLR and PLR were increasing, whereas MPV was decreasing in active jBD patients. PLR was a possible risk predictor for active disease in jBD. These parameters are easily accessible inflammatory markers that may help in the diagnosis and evaluaion of the disease activity of jBD, in early identification of various comorbidities and in monitoring the response to therapies. Further research is necessary to clarify the predictive role of PLR and inflammatory markers in jBD disease activity.

Conflict of interest
No conflict of interest was declared by the authors.

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