

Increased Mean Platelet Volume in Behçet's Disease with Thrombotic Tendency

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The relationship between Behçet's disease (BD) and platelet aggregation has not sufficiently been investigated yet. Mean platelet volume (MPV) is a marker of platelet function, and the increase in MPV has been identified as an independent risk factor of recurrent vascular events. BD is characterized by a relapsing vasculitis of the venous as well as arterial thrombosis. However, the precise pathogenic mechanisms underlying thrombotic tendency in BD are not known. We hypothesized that there might be an association between thrombotic complication and MPV in these patients. Therefore, we investigated activation of platelets in patients with BD using a simple marker, MPV, the most accurate measure of platelet size. A total of 60 patients with BD and 40 age- and gender-matched controls were included. The BD patients were divided into subgroups based on the presence ($n = 22$) or absence of thrombosis ($n = 38$) and clinically active ($n = 30$) or inactive ($n = 30$) state. MPV was higher in patients with BD than controls (8.14 ± 0.8 vs. 7.48 ± 0.3 fl, $p = 0.001$). Among BD patients, MPV was larger in patients with thrombosis than those without thrombosis (8.45 ± 1.0 vs. 7.96 ± 0.7 fl, $p = 0.038$). However, there was no significant difference in MPV between BD patients with active and inactive states. The increase in MPV is independent of the disease activity, and the presence of thrombosis is associated with higher MPV in BD patients. Therefore, antiplatelet therapy may be useful to prevent thrombotic complications in BD patients.

Keywords: mean platelet volume; Behçet's disease; platelet; thrombosis; vasculitis

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Behçet's disease (BD) is a systemic disorder characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis and skin lesions (O'Duffy 1990; Sakane et al. 1999; Yurdakul et al. 2004). Vascular involvement is a major clinical feature of BD. The incidence of vascular involvement has been reported between 7.7% and 43% in patients with BD (Koç et al. 1992; Ames et al. 2001; Yurdakul et al. 2004). Although venous thrombosis is a common clinical manifestation, the causes of venous thrombosis are still unknown in patients with BD (Kiraz et al. 2002; Yurdakul et al. 2004). However, the endothelial dysfunction is thought to play a key role for development of thrombosis in BD (Schmitz-Huebner and Knop 1984; Koşar et al. 2002; Ozdemir et al. 2004). Additionally, there is the hypercoagulable/prothrombotic state in BD, which is also important in development of thrombosis (Gül et al. 2001; Kiraz et al. 2002; Koşar et al. 2002). Although the coagulation system, fibrinolytic activity and thrombophilic factors were studied extensively in patients with BD, platelet function, which is a major component of hemostasis and thrombosis,

has not been evaluated sufficiently.

Mean platelet volume (MPV) reflects platelet function and activity (Martin et al. 1991; Smith et al. 1999; Park et al. 2002). Changes in platelet behavior, such as increased platelet aggregability, have been proved to be an independent risk factor for cardiovascular events (Martin et al. 1991; Park et al. 2002). Platelet production and stimulation indirectly elevates values, which can result in cardiovascular disease, as larger platelets are more reactive than normalized ones (Park et al. 2002; Chu et al. 2010). Taking into account the thrombotic complication in BD patients, we hypothesized that there might be an association between thrombotic complication and MPV in these patients. Therefore, we compared MPV in BD patients with that of healthy controls. We also analyzed the difference in MPV between BD patients with and without thrombosis and between patients with active and inactive disease states.

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Materials and Methods

Study group

A total of 60 patients with BD fulfilling the inclusion criteria (29 female and 31 male, mean 40.5 ± 12.9 years) and 40 controls (22 female and 18 male, mean 41.3 ± 6.8 , years) were included to the study. The diagnosis of BD was made according to the criteria of International Study Group. The control group consisted of sex- and age-matched healthy subjects. A detailed history was taken and each participant underwent a systemic physical examination to exclude cardiovascular or other relevant disease before attending to the study. The exclusion criteria were hematological disorder, impaired coagulation tests, hyperlipidemia, hypertension, smoking, diabetes mellitus, renal and hepatic failure, antiphospholipid antibody positivity, active infectious disease, malignancy immunological disease and chronic obstructive lung disease. None of the patients took oral anticoagulant drugs, aspirin and other antiplatelets drugs during the blood sampling. The 20 of the patients with BD were taking colchicine but not the other drugs. However, it was stopped at least 3 days before the blood sampling.

The BD patients were divided to subgroups based on the presence ($n = 22$) or absence of thrombosis ($n = 38$) and these patients were also classified as active ($n = 30$) or inactive ($n = 30$) state according to clinical findings depending on the presence of at least two of the following criteria: oral ulcers, genital ulceration, eye lesions, skin lesions, arthritis and thrombophlebitis. The diagnosis of vascular thrombosis was confirmed by clinical findings, Doppler ultrasonography and/or angiography. In addition to MPV, some hematological parameters such as erythrocyte sedimentation rate (ESR),

total platelet count, white blood cell count and C-reactive protein (CRP) were measured in all subjects. The study protocol was approved by the institutional ethics committee and all participants gave informed consent.

Laboratory analysis

All blood samples were drawn from the antecubital vein at 08.00-10.00 a.m. after a fasting period of 12 hours. Analyses were performed immediately after sampling to prevent in vitro platelet activation. MPV and the other hematological parameters were measured by using Beckman Coulter LH 780 Hematology Analyzer. The expected values for MPV in our hematology laboratory ranged from 6.8 to 10.8 fl. Serum CRP levels were measured by nephelometric method (Behring Nephelometer Analyzer, Germany) and ESR was also measured by Westergren method. The reference ranges for CRP and ESR are 0 to 3.13 mg/l, and 1 to 20 mm/h, respectively.

Statistical analysis

Statistical analysis was performed by using the SPSS for windows 11.0 (Chicago, Illinois, USA). Data were expressed as mean \pm s.d. and nominal parameters were expressed as percent. For continuous variables, unpaired Student *t* test or Mann-Whitney-*U* test and for categorical changes, chi-square test was used. Correlation analysis between MPV and other variables was performed by the Pearson and Spearman rank correlation tests where appropriate. A *p* value < 0.05 was considered to indicate statistical significance.

Table 1. Baseline demographic properties of BD patients.

Parameters	Behçet disease ($n = 60$)	Thrombosis (+) ($n = 22$)	Thrombosis (-) ($n = 38$)
Female: male	29/31	5/17	24/14
Age, years	40.5 ± 12.9	45.3 ± 5.7	41.2 ± 4.5
Disease duration (years)	9.1 ± 5.1	9.6 ± 4.0	8.8 ± 5.6
Oral ulcerations	60 (100%)	22 (100%)	38 (100%)
Genital ulcerations	52 (86.7%)	19 (86.4%)	33 (86.8%)
Skin lesions	41 (68.3%)	15 (68.2%)	26 (68.4%)
Eye involvement	22 (36.7%)	3 (13.6%)	19 (50%)
Arthritis	43 (71.7%)	16 (72.7%)	27 (71.1%)
Positive pathergy test	15 (25%)	4 (18.2%)	11 (28.9%)
Central nervous system involvement	34 (56.7%)	13 (59.1%)	21 (55.3%)

Table 2. Comparison of the laboratory parameters of BD patients and controls.

Parameters	Behçet disease ($n = 60$)	controls ($n = 40$)	<i>p</i> value
MPV (fl)	8.14 ± 0.88	7.48 ± 0.37	$p < 0.001$
Total platelet count ($\times 10^9/l$)	298 ± 89	314 ± 67	NS
WBC ($\times 10^9/l$)	9.01 ± 2.7	7.76 ± 1.8	$p = 0.01$
ESR (mm/h)	33.03 ± 12.8	11.76 ± 3.5	$p = 0.01$
CRP (mg/l)	25.22 ± 11.9	4.56 ± 2.5	$p < 0.001$

WBC, White blood cell; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; and NS, statistically not significant.

Table 3. Comparison of the laboratory parameters of BD patients with and without thrombosis.

Parameters	Thrombosis (+) (n = 22)	Thrombosis (-) (n = 38)	p value
MPV (fl)	8.45 ± 1.0	7.96 ± 0.7	p = 0.038
Total platelet count (× 10 ⁹ /l)	293 ± 83	306 ± 78	NS
WBC (× 10 ⁹ /l)	9.35 ± 2.3	8.81 ± 2.9	p = 0.45
ESR (mm/h)	56.86 ± 19.2	36.88 ± 10.2	p < 0.001
CRP (mg/l)	49.68 ± 17.5	15.27 ± 4.2	p < 0.001

Table 4. Comparison of the laboratory parameters of BD patients at active and inactive states.

Parameters	Active BD (n = 30)	Inactive BD (n = 30)	p value
MPV (fl)	8.20 ± 0.6	8.08 ± 1.0	0.601
Total platelet count (× 10 ⁹ /l)	295 ± 85	303 ± 82	NS
WBC (× 10 ⁹ /l)	9.23 ± 3.2	8.79 ± 2.0	0.52
ESR (mm/h)	36.43 ± 5.6	29.63 ± 4.9	0.009
CRP (mg/l)	25.49 ± 14.6	24.95 ± 10.8	0.426

Results

There was no significant difference between BD and controls in terms of baseline demographic properties. Among 22 BD patients with vascular thrombosis, 17 were male patients, and the distribution of thrombi was in vena cava superior (n = 1), vena cava inferior (n = 3) and deep vein thrombosis (n = 18), respectively. Baseline demographic properties of BD patients are shown in Table 1. MPV was higher in patients with BD than that in controls (8.14 ± 0.8 vs. 7.48 ± 0.3 fl, p = 0.001, respectively) (Table 2). The other laboratory parameters are also listed in Table 2.

Among BD patients, MPV was higher in patients with thrombosis than the value in those without thrombosis (8.45 ± 1.0 vs. 7.96 ± 0.7 fl, p = 0.038, respectively) (Table 3). In contrast, there was no significant difference in MPV between BD patients with active state and those with inactive state (8.20 ± 0.6 vs. 8.08 ± 1.0 fl, p = 0.6, respectively) (Table 4). Correlation analysis showed no significant association between MPV and any other parameter.

Discussion

Principle findings of this study were 1) MPV was significantly higher in patients with BD when compared with that of controls, 2) MPV was higher in BD patients with thrombosis than those without thrombosis, and 3) there was no significant difference in MPV between BD patients in active and inactive state. To our knowledge, this is the first study showing the increased MPV in patients with BD.

BD is a multisystemic vasculitis with obscure etiology. Thrombotic complications have been reported in approximately 12-40% of patients with BD (Koç et al. 1992). Venous thrombosis, especially deep vein thrombosis of lower extremity, is the common clinical manifestation in vascular

BD (O'Duffy 1990; Koç et al. 1992). Venous thrombosis in BD is mainly observed in males (O'Duffy 1990; Koç et al. 1992; Kiraz et al. 2002; Duzgun et al. 2006). In our study, the characteristics of BD patients with venous thrombosis were similar to previous studies. The exact pathogenic mechanism underlying the venous thrombosis is still unclear. However, vascular endothelial cell injury and resultant endothelial dysfunction are thought to play a central role in the development of thrombosis (Schmitz-Huebner and Knop 1984; Kiraz et al. 2002; Koşar et al. 2002; Ozdemir et al. 2004; Duzgun et al. 2006). Investigations of the hemostatic parameters have shown activation of both coagulation and fibrinolytic systems suggesting endothelial activation or injury. Kiraz et al. (2002) reported that procoagulant markers of thrombosis reflecting intravenous excessive thrombin formation, defective fibrinolysis and hypercoagulable/prothrombotic state and endothelial injury were associated thrombotic complication in BD. Akar et al. (2006) evaluated circulating activated platelets and platelet reactivity in BD and demonstrated that platelets in BD patients were active and more reactive than normal controls and concluded that this might contribute to the thrombotic tendency. Yapişlar et al. (2007) suggested decreased nitric oxide and increased platelet aggregation in patients with BD, particularly in the inactive period and concluded that the risk of thrombosis was independent of the disease activity phase. In accordance with these reports we found that MPV values were increased in BD patients particularly with thrombosis independent of the disease activity.

Platelets play an important role in the process of hemostasis and thrombosis. Following injuries to blood vessels and in certain pathologic conditions, platelets adhere to the exposed subendothelial connective tissue, aggregate and release several biologically active substances (Chiang et al.

2002). Alteration in platelet aggregation is the main event leading to imbalance in hemostasis, which may eventually lead to thrombotic disorders. In addition, platelet aggregation may contribute to the vasculitis occurring in BD (Singh et al. 2002). Accordingly, MPV is a marker of platelet function and large platelets contain more dense granules and produce more thromboxane A₂ (Kamath et al. 2001; Park et al. 2002). Increased MPV has been associated with greater in vitro aggregation in response to ADP and collagen (Kamath et al. 2001). Increased platelet size has been reported in patients with vascular risk factors such as diabetes, smoking and acute ischemic stroke (Tschoepe et al. 1991; Kario et al. 1992; Smith et al. 1999). Therefore, increased MPV could be regarded as a marker for development of thrombosis in BD.

There is no optimal approach for the treatment of venous thrombosis in BD. The treatment modalities include immunosuppressive agents such as azathioprine, aspirin and anticoagulation for deep vein thrombosis. However, the use of anticoagulants or anti-platelet agents in the management of thrombosis in BD is controversial. The venous thrombi in BD adhere to the vessel wall but do not result in emboli, and pulmonary embolism is rare despite a high frequency of venous thrombosis and thus anticoagulants are not recommended (Hatemi et al. 2008). Ahn et al. (2008) reported that immunosuppressive therapy, rather than anticoagulation therapy, is essential for the treatment of deep venous thrombosis associated with BD. Regarding the use of aspirin, while some have recommended the others does not (Sakane et al. 1999; Yazıcı et al. 2007; Yapişlar et al. 2007; Hatemi et al. 2008). From the clinical point of view, only depending on increased MPV values, it is difficult to claim that anti-platelet and anticoagulant agents should be given to all BD patients. However, at least it should be kept in mind that together with defective fibrinolysis, hypercoagulable/prothrombotic state and endothelial injury, increased MPV values may be one of the parameters facilitating thromboembolic complication in these patients

Study limitations

The main limitation of our study was lack of the other markers of platelet activation and aggregation such as platelet factor 4 and beta thromboglobulin, thrombotic status (fibrinopeptide A and thrombin-antithrombin III complex) and fibrinolytic status (D-dimer and plasmin-alpha 2-plasmin inhibitor complex). The other limitation was the fact that only small number of patients were included.

Conclusion

Patients with BD have higher MPV, indicating the tendency toward platelet aggregation, when compared to controls, and the presence of thrombosis is associated with higher MPV in these patients. However, further large-scale studies are required to clarify whether the BD patients with high MPV are at greater risk for thrombotic complications and may benefit from the anti-platelet therapy.

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