Evaluation of Mean Platelet Volume in Patients With Behcet’s Disease as an Indicator of Vascular Thrombosis

Hatice Ata, MD1; Filiz Canpolat, MD1; Fatma Eskioglu, MD1

University of Health Sciences, Dıkapı Yıldırım Beyazıt Training and Research Hospital, Department of Dermatology, Ankara, Turkey

Abstract
Background: Patients with Behcet’s disease (BD) are recognized with increased risk for venous and/or arterial thrombosis. Thrombotic tendency of BD is not known. Vascular injury, loss and dysfunction/hyperfunction of endothelial cells are believed to play a role in thrombosis development. Injury and inflammation due to vasculitis can cause platelet response with increase in mean platelet volume (MPV) and thrombosis in BD. In this study, we aimed to compare the levels of MPV between patients with BD and healthy controls, and also show its effect on thrombosis.

Methods: One hundred patients with BD and 100 healthy controls were evaluated for MPV levels with clinical findings in age-gender matched case-control study. The variables of patients and controls were compared and correlated using chi-square, Mann-Whitney U and Spearman tests. Logistic regression analysis was used to determine independent predictors of vascular involvement and thrombosis.

Results: Mean MPV was significantly higher in patients with BD than healthy controls (MPV; Patients: 9.2 ± 0.9 [7.3–12.9] vs. Controls: 8.2 ± 0.6 [6.8–10.6] fl; P < 0.0001). Platelets levels were lower than controls, but not significantly (236 ± 52.3 [112–451] vs. 245 ± 52.8 [141–467] x10^9/L, P = 0.55). Negative correlation was found between platelet count and MPV in patients (r = -0.51, P = 0.01). Presence of erythema nodosum (EN) and MPV were determined as predictors for vascular involvement and thrombosis (EN: P < 0.0001, OR [95% CI] = 35.4 [6.3–178.2]; MPV: P < 0.0001, OR [95% CI] = 12.8 [4.1–24.3]).

Conclusion: MPV is a simple measurement for indirect monitoring of platelet activity and thrombotic potential. MPV and EN may be independent risk factors for vascular thrombosis in BD. Patients with higher MPV levels and EN in BD, might have been pursued closely for enhancing thrombosis. We advise to check the MPV and put the patients on anticoagulation if it is high.

Keywords: Behcet’s disease, Mean platelet volume, Thrombosis, Vascular


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Introduction
Behcet’s disease (BD) is a kind of variable vessel vasculitis with unknown aetiology characterized by oral or genital aphthous ulcerations and uveitis.1–3 Vascular thrombosis is one of the hallmarks of this disease. It may occur both in veins and arteries, but in the literature, venous involvement with varying ratios is reported nearly 6 times higher in cases with BD in large series of studies (13.8% vs. 2.4%).4 It occurs especially in deep or superficial veins of the lower extremities.

Pathogenesis of thrombosis in BD is not completely understood, but various mechanisms have been suggested for the increased risk of thrombosis in BD. Impaired fibrinolysis, increased clotting factors, and abnormal platelet function are some of them. BD should be evaluated as a hypercoagulable/prethrombotic state due to tendency to the thrombosis.5–8 Platelets play an important role in the integrity of normal homeostasis. Mean platelet volume (MPV) is the indicator for platelets function. The large platelets which contain more dense granules are more potent and thrombogenic than smaller platelets.6 Vascular risk factors such as hypercholesterolemia, diabetes mellitus (DM) and hypertension may contribute to MPV increase. Conflicting results are presented in the literature regarding increased and decreased levels of MPV in BD.5–8 In this study, due to hypercoagulable/prethrombotic state with high incidence rate of thrombosis, we studied MPV and thrombosis in BD. We aimed to compare the levels of MPV between patients with BD and healthy controls, and show the effect of MPV and activity of disease on thrombosis.

Material and Methods
Subjects
This was an age-gender matched case-control study. One hundred patients with BD who were diagnosed according to the International Criteria for BD (ICBD)9 and admitted to the outpatient clinic of dermatology for medical examinations (52 female, 48 male with a mean age of 39.7 ± 11.1), and 100 healthy age-gender matched controls (54 female, 46 male, with a mean age of 39.2
Patients with BD are recognized with increased risk for venous and/or arterial thrombosis. Therefore, it has been termed as a hypercoagulable prethrombotic state. The exact pathogenic mechanism investigated using analytic methods to determine whether or not they are normally distributed. Descriptive analyses were done using mean ± standard deviation (SD). For discontinuous variables such as gender, chi-square test was used. For continuous variables such as MPV and platelet counts of patients and controls, Mann-Whitney U test was used. Correlation analysis was performed using Spearman test. Factors such as age, gender, activity of BD, onset and duration of BD, percentage of oral aphthous ulcers, genital ulcers, papulopustular eruptions, involvement of eyes, involvement of vascular regions, EN, pathergy positivity, using a treatment such as colchicine, cyclosporine, steroid, interferon with P value less than 0.2 shown by univariate analyses to be associated with vascular involvement were further entered into the multivariate analysis of logistic regression analysis to determine independent predictors of vascular involvement and thrombosis. A P value of less than 0.05 was considered to show a statistically significant result.

**Results**

The demographic features were summarized in Table 1. Mean MPV was significantly higher in patients with BD than healthy controls (9.2 ± 0.9 [range: 7.3–12.9] vs. 8.2 ± 0.6 [range: 6.8–10.6] fl; P < 0.0001). Platelets levels were lower than controls, but there was no significant difference (236 ± 52.3 [range: 112 – 451] vs. 245 ± 52.8 [range: 141–467] ×10^11/L, P = 0.55). A negative correlation was found between platelet count and MPV in patients (r = 0.51, P = 0.01) (Table 2). Presence of EN and MPV were determined as predictors for vascular involvement and thrombosis (EN: P < 0.0001, OR [95% CI] = 35.4 (6.3–178.2); MPV: P < 0.0001, OR [95% CI] = 12.8 [4.1–24.3]) (Table 3). Mean MPV was 8.5 ± 0.5 (Range: 7.3-9.1) fl in patients with EN (n = 7) and 9.2 ± 0.8 (range: 7.6–12.9) fl in patients without EN (n = 93), P = 0.019. MPV was 9.8±1.2 (range: 8.7-12.9) fl in patients with vascular involvement (n = 15) and 9.1 ± 0.5 (range: 7.3-11.1) fl in others (n=85), P = 0.017. Twenty-seven percent of patients (n = 27) had active disease(s). Mean MPV was not found significant between active (n = 27) and inactive disease(s) (n = 73), respectively (MPV: 9 ± 1.1 [Range: 7.7–11.1] fl vs. 9.3 ± 0.8 [range: 7.6–12.9] fl P = 0.44). Other parameters such as status of pathergy test, treatment use, other site involvement and past history features were not significant for MPV (P > 0.05).

**Discussion**

BD is a relapsing vasculitis in which orogenital ulceration is a prominent feature. Patients with BD are recognized with increased risk for venous and/or arterial thrombosis. Therefore, it has been termed as a hypercoagulable prethrombotic state. The exact pathogenic mechanism

**Activity of Disease**

Activity of disease was determined by both clinical and laboratory findings. In clinical evaluation, patients who had worsening clinical symptoms at the time of the study, and also had three of the major criteria (oral ulcers, genital ulceration, eye lesions, skin lesions, and positive pathergy test) were considered to be in the active period of the disease.

Presence of thrombosis: Thrombosis was confirmed with clinical findings, Doppler ultrasonography and/or angiography.

**Laboratory Analysis**

Blood samples were drawn after a fasting period of 12 hours. MPV was collected in tubes with citrate in order to rule out the formation of platelet aggregation with ethylenediaminetetraacetae (EDTA). Blood samples were centrifuged within one hour after sampling. Beckman Coulter LH 700 Haematology Analyser based on light scattering impedance and conductivity (VCS technology) was used to measure complete blood cell count and other haematological parameters. The normal range of MPV in our haematology laboratory was 7.2 to 10.8 fl.

**Statistical Analysis**

Statistical analyses were performed using SPSS software, version 15. The variables of patients and controls were
Table 1. Demographic Features of Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Gender, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52 (52)</td>
<td>54 (54)</td>
<td>0.86</td>
</tr>
<tr>
<td>Male</td>
<td>48 (48)</td>
<td>46 (46)</td>
<td></td>
</tr>
<tr>
<td>Age (y), mean ± SD (range)</td>
<td>39.7 ± 11.1 (18–66)</td>
<td>39.2 ± 11.3 (18–62)</td>
<td>0.83</td>
</tr>
<tr>
<td>Beginning age of disease, y</td>
<td>30.6 ± 7.7 (16–50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of disease, y</td>
<td>9.1 ± 6.6 (0–30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV, fl</td>
<td>9.2 ± 0.9 (7.3–12.9)</td>
<td>8.2 ± 0.6 (6.8–10.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Platelets, × 10^9/L</td>
<td>216 ± 52.3 (112–451)</td>
<td>245 ± 52.8 (141–467)</td>
<td>0.55</td>
</tr>
<tr>
<td>Presence of aphthous lesion, No. (%)</td>
<td>67 (67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of genital ulcer, No. (%)</td>
<td>12 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papulopustular eruption, No. (%)</td>
<td>22 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of erythema nodosum, No. (%)</td>
<td>7 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of ocular involvement, No. (%)</td>
<td>4 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of joint involvement, No. (%)</td>
<td>60 (60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of vascular involvement, No. (%)</td>
<td>15 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of neurological involvement, No. (%)</td>
<td>7 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of gastrointestinal involvement, No. (%)</td>
<td>8 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of genitourinary involvement, No. (%)</td>
<td>3 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undergone systemic treatment, No. (%)</td>
<td>96 (96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathergy test positivity, No. (%)</td>
<td>21 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of active disease, No. (%)</td>
<td>27 (27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; MPV, mean platelets volume.

Table 2. Significant Linear Correlation Between MPV and Platelets in Patients With BD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV-platelets</td>
<td>-0.51</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviation: BD, Behcet’s disease; MPV, mean platelets volume. P<0.05 is significant.

Table 3. Effects of Some Predictors on Vascular Involvement and Thrombosis in Univariate and Multivariate Analyses

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Reference Category</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in age</td>
<td></td>
<td>P</td>
<td>OR</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>0.42</td>
<td>0.94</td>
</tr>
<tr>
<td>Increase in duration of disease</td>
<td></td>
<td>0.24</td>
<td>2.4</td>
</tr>
<tr>
<td>Increase in MPV</td>
<td></td>
<td>0.85</td>
<td>1.1</td>
</tr>
<tr>
<td>Increase in platelet</td>
<td></td>
<td>&lt; 0.0001</td>
<td>7.6</td>
</tr>
<tr>
<td>Presence of aphthous lesion</td>
<td>Yes</td>
<td>0.61</td>
<td>0.98</td>
</tr>
<tr>
<td>Presence of genital ulcer</td>
<td>Yes</td>
<td>0.49</td>
<td>1.8</td>
</tr>
<tr>
<td>Presence of papulopustular eruption</td>
<td>Yes</td>
<td>0.98</td>
<td>0.97</td>
</tr>
<tr>
<td>Presence of ocular involvement</td>
<td>Yes</td>
<td>0.41</td>
<td>1.9</td>
</tr>
<tr>
<td>Presence of joint involvement</td>
<td>Yes</td>
<td>0.37</td>
<td>3.1</td>
</tr>
<tr>
<td>Presence of neurological involvement</td>
<td>Yes</td>
<td>0.25</td>
<td>2.6</td>
</tr>
<tr>
<td>Presence of gastrointestinal involvement</td>
<td>Yes</td>
<td>0.54</td>
<td>2.1</td>
</tr>
<tr>
<td>Presence of genitourinary involvement</td>
<td>Yes</td>
<td>0.71</td>
<td>1.5</td>
</tr>
<tr>
<td>Pathergy test positivity</td>
<td>Yes</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>Pathergy test positivity</td>
<td>Yes</td>
<td>0.33</td>
<td>2.2</td>
</tr>
<tr>
<td>Presence of active disease</td>
<td>Yes</td>
<td>0.79</td>
<td>0.8</td>
</tr>
<tr>
<td>Presence of erythema nodosum</td>
<td>Yes</td>
<td>&lt; 0.0001</td>
<td>11.3</td>
</tr>
<tr>
<td>Undergone treatment</td>
<td>Yes</td>
<td>0.75</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Abbreviation: MPV: mean platelets volume. P<0.05 = significant; CI: confidence intervals were estimated using logistic regression model; OR: odds ratio was estimated using logistic regression model. Simple: first was selected to evaluate the effect of the categorical predictors.

underlying thrombotic tendency of BD is still unknown. A number of mechanisms have been implicated to account for high incidence of thrombosis. Endothelial cell injury and resultant endothelial loss and/or dysfunction/hyperfunction seem to be some key events in the prethrombotic state of BD. Normal haemostatic
response is initiated when endothelial damage disrupts
the vascular endothelial lining, and blood is exposed to
subendothelial connective tissues. Hypofibrinolysis and
defective fibrinolytic response are proposed as a part of
generalized cell dysfunction in BD.
Platelets have a central role in the pathogenesis of
thromboembolic diseases. In BD, platelets are active
and more reactive than normal controls which may
contribute to the thrombotic tendency. It could be
speculated that three factors may contribute to increased
platelet activation: both arterial and venous wall injuries,
circulating inducers of platelet activation, and genetic
predisposition. However, the difference in platelets levels
between patients and controls is clinically unimportant.

MPV is an important biological variable. Large platelets
have higher thrombotic potential. In comparison to
smaller ones, larger platelets are denser, aggregate more
rapidly with collagen, have higher thromboxane A2
level and express more glycoprotein Ib and IIb/IIIa
receptors. An increased MPV decreases the inhibitory
effectiveness of prostacyclins on platelets aggregation
and release reaction.

Increase in MPV is now emerging as an independent
risk factor for thromboembolism, stroke and myocardial
infarction. Also increased levels of MPV are identified as a predictor for venous thromboembolism of
unprovoked origin.
Venous thrombosis in BD might be due to vasculitis
itself or hypercoagulable status whether venous thrombi
seem to be caused by inflammation of the vessel wall
rather than by a primary clotting abnormality. Systemic
immunosuppressive agents are used in an effect to
prevent this inflammation. Guideline on thrombosis in
BD comes from a recent small study which compares the
use of immune suppression and anticoagulation. In this
study, the authors suggested that using anticoagulation
in BD patients with thrombosis may be unnecessary.
Another study showed that taking anticoagulants may
be appropriate, at least until the diagnosis is confirmed
and inflammation is controlled. Whereas, according
to the latest European League Against Rheumatism
(EULAR) Guidelines for treatment of BD, the use of
anticoagulation is forbidden unless it is proved that
the patient has no tendency for hemorrhage and
aneurism. In our opinion, anticoagulant agents may
be used because of high coagulation tendency, until the
disease is controlled. Decreasing MPV may indicate the
control of the disease.

However, the potential contribution of MPV to
development of thrombosis in BD is unclear. Association
between BD and MPV levels has been demonstrated in
two previous studies with conflicting results. Different
factors such as time from venepuncture, choice of
anticoagulant, sample storage temperature, and/or
equipment for measuring MPV can give different
results. On the other hand, some patients are on
treatment for BD which might affect the results. In
our study, we found increased MPV levels in patients
with BD. In addition, MPV was found significantly
higher in BD patients with thrombosis than those
without thrombosis. Increased MPV levels may have an
association with thrombosis due to large thrombogenic
platelets and vascular involvement of disease. In this
study, increase in MPV in patients with BD was related
to a 12.8-fold increased risk for vascular involvement
and thrombosis in multivariate analysis (OR = 12.8,
P < 0.0001). This might be associated with chronic or
controlled inflammatory processes due to vasculitis.

Increased MPV in inflammatory status contributes to
thrombosis.

There was no difference between active patients and
inactive patients with BD for MPV. Some studies show
that low MPV and high platelet levels are found in acute
course of Familial Mediterranean Fever, ankylosing
spondylitis, and rheumatoid arthritis. Cytokines such as
IL-1, 3, 4, 6, 11 and TNF-alpha can contribute to this
fact. In addition, suppression of acute proinflammatory
status of disease can contribute to increase of MPV.
In our study, we found decreased levels of MPV in EN.
This result and negative correlation between MPV and
platelets supported the decreased levels of MPV in acute
conditions. However, presence of EN in patients with
BD was related to a 35.4-fold increased risk for vascular
involvement and thrombosis in multivariate analysis (OR
= 35.4, P < 0.0001). Inflammatory status of BD is very
complex with acute and chronic processes. To sump up,
presence of EN and increased MPV levels were found as
independent predictors for thrombosis in BD.

Unfortunately, the main limitation of this study is
that, there is not any control with other acute, chronic
inflammatory and infection diseases to compare to our
subjects with BD. In addition, the BD with acute deep
vein thrombosis (DVT) could be compared to acute and
chronic DVT due to other causes.

In conclusion, MPV which is one of the markers
indicating the function of platelets is a simple and easy
measurement of indirect monitoring of platelet activity
and thrombotic potential. In our study, the significance
of MPV was evaluated in BD because of the fact that
many studies about MPV had been studied on venous
thromboembolism, other vascular diseases (e.g stroke)
and vascular disease risk factors such as obesity, DM
and metabolic syndrome. Most of our patients were on
systemic treatments which could be a limitation in our
study. Increased level of MPV was found in patients with
BD compared to controls. It may be an independent risk

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factor for vascular involvement and thromboembolism in BD. BD patients who have higher MPV levels must be pursued closely for thrombosis. Decreased or increased levels of MPV can indicate disease palliation or progression, respectively. Antiocoagulant agents can be used due to high coagulation tendency until the disease is controlled. According to the latest EULAR Guidelines for the treatment of BD, the use of antiocoagulation is forbidden unless it is proved that the patient has no tendency for hemorrhage and aneurism. However, we advise to check MPV and put the patient on antiocoagulation if it is high, and continue until it reaches the normal level. We also suggest further studies to evaluate the role of MPV on thrombosis in BD.

Authors' Contribution
Study design: HA, FC, FE. Study conduct: HA, FE. Data collection: HA. Data analysis: HA. Data interpretation: HA, FC, FE. Drafting of the manuscript: HA, FC, FE. Revising manuscript content: HA, FC, FE. Approving final version of manuscript: HA, FC, FE. HA takes responsibility for the integrity of the data analysis.

Conflict of Interest Disclosures
The authors have no conflicts of interest.

Ethical Statement
Ethical approval and informed consent were obtained before beginning the study.

References
29. Ahn JK, Lee YS, Jeon CH, Koh EM, Cha HS. Treatment of venous thrombosis associated with Behcet's disease: immunosuppressive therapy alone versus immunosuppressive...