

Original Article

Plasma Homocysteine Level in Patients with Behcet's Disease with or without Thrombosis

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Abstract

Aim: To find the possible role of plasma homocysteine level as a contributing factor in venous and arterial thrombosis in patients with Behcet's disease (BD).

Methods: In a case control study, two groups of BD patients were included: 47 with thrombosis and 49 without thrombosis. All patients fulfilled the International Study Group Criteria for BD and the confirming diagnostic procedures for vascular thrombosis were either Doppler sonography or angiography. Forty-nine controls were selected by consecutive sampling among age and sex matched healthy subjects. Plasma homocysteine level was measured by ELISA in all. The clinical and laboratory characteristics of the disease were compared between the two groups of BD patients. Comparisons were done by ANOVA and Chi square tests; correlations were analyzed with Pearson test.

Results: The mean plasma homocysteine level was significantly higher in BD patients (14.9 ± 13.9 $\mu\text{Mol/L}$) than in healthy controls (9.9 ± 6.7 $\mu\text{Mol/L}$), $P < 0.02$. The difference was also significant when comparing the three groups by ANOVA: BD patients with thrombosis (24.2 ± 13.2 $\mu\text{Mol/L}$), BD patients without thrombosis (5.9 ± 7.0 $\mu\text{Mol/L}$), and healthy controls ($P < 0.0001$). We found no correlation between plasma homocysteine level and any organ involvement other than thrombosis. The mean plasma homocysteine level was lower in HLA-B51 positive BD patients (11.6 ± 12.1 vs. 21.7 ± 16.3 $\mu\text{Mol/L}$, $P < 0.05$), but the difference was not significant in those with thrombosis (20.9 ± 13.2 vs. 29.5 ± 12.7 $\mu\text{Mol/L}$, $P = 0.18$).

Conclusion: Hyperhomocysteinaemia may be an independent risk factor for vascular thrombosis in patients with BD. This is the first study showing a negative correlation between HLA-B51 and plasma homocysteine level.

Keywords: Behcet's disease, HLA B51, homocysteine, thrombosis

Introduction

Behcet's disease (BD) is a multi-system inflammatory disorder of unknown etiology. Vascular thrombosis is one of the hallmarks of this disease,¹ and may be seen in 8.3% of Iranian patients² and in 7 to 38% of BD patients in other parts of the world.^{3,4}

It may occur both in veins and arteries, but venous involvement is much more common including more than 90% of cases,⁵ especially in deep or superficial veins of the lower extremities.^{2,6} Thrombosis may be a poor prognostic sign in BD when major vessels are involved.^{5,7}

The exact mechanism of this hypercoagulability state is not known. However, it may be due to both coagulation disorders and endothelial cell injuries.⁴ Although the primary reason for clot formation seems to reside in the inflammatory process in the arterial wall, various coagulation disorders were reported in patients with BD.⁸ Risk factors include protein C or S deficiency,⁹⁻¹² decreased anti-thrombin III

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level,¹⁰ presence of anti-phospholipid antibodies,¹³⁻¹⁵ increased thrombomodulin,^{4,16} mutation of factor V Leiden gene,^{6,17} dyslipidemia (particularly hypertriglyceridemia),¹⁸ and an increased serum homocysteine level.¹⁹⁻²²

Homocysteine is an intermediate amino acid formed during the conversion of methionine to cysteine. It has been suggested as a correctable risk factor for vascular disorders due to its probable mechanisms to induce thrombosis.^{21,23} These mechanisms include acceleration in low density lipoprotein oxidation,²⁴ antagonistic effects to nitric oxide as a vasodilating agent,²⁵ decreased circulation due to vasodilatation defects, platelet activation,²⁶ and pro-inflammatory response via IL-8 induction.²³

The aim of this study was to find the possible role of plasma homocysteine level as a contributing risk factor in venous and arterial thrombosis in patients with BD.

Patients and Methods

This case control study was done in patients with BD attending the Behcet's Disease Outpatient Clinic (Rheumatology Research Center, Shariati Hospital) during an 18 month period. All with a positive past history of vascular thrombosis (at least earlier than six months before referral) were included as the first patient group by consecutive sampling. The confirming diagnostic procedures were either Doppler sonography or angiography (conventional in earlier, and CT or MR angiography in recent years). For each case another BD patient without thrombosis was included on the same day in the same clinic. All patients fulfilled the International Study Group Criteria for Behcet's Disease.²⁷ The exclusion criteria for these patients were: the consumption of methotrexate, folic acid, or B12; malnutrition or body mass index (BMI)

less than 19; alcohol consumption, presence of chronic diseases such as diabetes mellitus, renal failure, cardiovascular diseases, thyroid dysfunction, viral hepatitis, psoriasis, cancers, inflammatory bowel disease (IBD), and other rheumatic diseases. Control selection was by consecutive sampling among age and sex matched healthy blood donors from the Iranian Blood Transfusion Organization.

Plasma homocysteine levels were determined by enzyme immunoassay (Axis® Homocysteine EIA, IBL-Hamburg, Germany) in all. This was done on 10 mL of the whole blood sample, drawn from a peripheral vein using a 25 gauge needle, after a 12-hour fast. The blood samples were collected in two ethylene diamine tetra acetate (EDTA) containing tubes (one as backup) and kept on ice in order to prevent false elevation due to homocysteine leakage from erythrocytes. They were centrifuged within one hour after sampling. A plasma homocysteine level in the range of 5 – 15 µMol/L was considered normal by this method. Different clinical and laboratory characteristics of the disease were determined in patients with BD.

Statistics

Comparisons were done by ANOVA test for continuous variables and between three groups (such as age and homocysteine level), and by Chi square test for discrete variables and between two groups. Correlation analysis was performed by the Pearson correlation test. A standard deviation (SD) for the means and a confidence interval at 95% (CI) was calculated for each item. *P* values of less than 0.05 were considered to be significant.

Ethics

The study protocol had been approved by the Eth-

Table 1. Comparison of demographic characteristics and homocysteine level between three study groups

	Behcet's disease with thrombosis	Behcet's disease without thrombosis	Control patients	<i>P</i> -value (ANOVA)
Patients number	47	49	49	—
Male/female ratio	33/14	25/24	38/11	<0.05
Mean age* (SD)†	42.4±10.9	38.3±9.6	39.7±11.4	>0.18
Homocysteine level‡ (SD)	24.2±13.2	5.9±7	9.9±6.7	<0.0001
*Years ; †Standard deviation ; ‡µMol/L				

Table 2. Comparison of clinical characteristics between two groups of Behcet's disease patients (with and without thrombosis)

	With thrombosis	Without thrombosis	P-value
Patients number	47	49	—
Oral aphthosis*	95.3	98	0.60
Genital aphthosis*	76.7	67.3	0.99
Skin lesions*	72.1	65.3	0.49
Ocular lesions*	32.6	59.2	<0.02
Joint involvement*	34.9	30.6	0.19
CNS involvement*	14	0	0.009

*Percentage

Table 3. Comparison of laboratory results between two groups of Behcet's disease patients (with and without thrombosis)

	With thrombosis	Without thrombosis	P-value
Patients number	47	49	—
High ESR*	46.5	51	0.19
Positive pathergy test*	48.8	59.2	0.29
HLA-B5*	44.2	57.1	0.18
HLA-B51*	18.6	22.4	0.31
HLA-B27*	4.7	8.2	0.68

* Percentage

ic's Committee of Tehran University of Medical Sciences, and a written informed consent was obtained from each participant.

Results

Forty-seven BD patients with thrombosis and 49 without thrombosis were enrolled. The control group included 49 healthy blood donors. Table 1 shows the demographic characteristics and homocysteine levels in these three groups. The one-way ANOVA test showed no significant difference between the three groups in the mean age ($P>0.18$), while the difference was significant for sex ($P<0.05$). While post hoc analysis showed the difference was not significant in the sex ratio between BD patients with thrombosis

and the two other groups ($P>0.09$), whereas it was significant when comparing the BD group without thrombosis and control group ($P<0.004$).

The mean plasma homocysteine level was significantly higher in BD patients in comparison to healthy controls (14.9 ± 13.9 versus 9.9 ± 6.7 $\mu\text{Mol/L}$, $P<0.02$). The difference was also significant when comparing the subgroups of BD patients with thrombosis to healthy controls (24.2 ± 13.2 versus 9.9 ± 6.7 $\mu\text{Mol/L}$, $P<0.001$). The mean plasma homocysteine level was significantly lower in the subgroup of BD patients without thrombosis than in those with thrombosis ($P<0.001$) while non-significantly lower than healthy controls ($P=0.09$), (Table 1).

In BD patients, the homocysteine level was significantly higher in males (17.3 ± 14.4 $\mu\text{Mol/L}$) than in fe-

Table 4. Comparison of the mean plasma homocysteine levels in 92 patients with Behcet's disease according to different disease characteristics

Disease characteristics	Positive		Negative		P-value
	Number	Homocysteine level*	Number	Homocysteine level*	
Skin lesions	43	12.2±13.3	49	16.4±14.1	0.16
Ocular lesions	63	14.5±13.5	29	14.3±14.6	0.42
Joint involvement	30	14.5±13.6	62	14.4±14.0	0.67
CNS involvement	6	20.6±12.8	86	14.0±13.8	0.87
High ESR	45	13.4±14.4	47	15.4±13.2	0.90
Positive pathergy test	50	14.2±13.9	40	15.2±14.1	0.88
HLA-B5	47	13.4±13.7	35	17.0±14.8	0.25
HLA-B51	19	11.6±12.1	11	21.7±16.3	0.004
HLA-B27	6	15.8±16.8	66	15.4±14.4	0.64

*Mean±standard deviation, $\mu\text{Mol/L}$

males ($11.1 \pm 12.4 \mu\text{Mol/L}$) at $P < 0.04$, but the difference was not significant between males and females with thrombosis ($25.1 \pm 13.7 \mu\text{Mol/L}$ in males versus $22 \pm 12.1 \mu\text{Mol/L}$ in females, $P = 0.46$).

The involved vessels were deep vein thrombosis in 38 patients, superficial phlebitis in four, cavernous sinus and superior vena cava each in one patient. Abdominal aortal thrombosis was seen in three cases; one with aneurysm and one with dissection and embolism.

The frequency of different organ involvements was generally higher in patients with thrombosis, but this difference was statistically significant only in the case of CNS involvement ($P = 0.009$, Table 2). The frequency of ocular lesions was significantly lower in those with thrombosis ($P < 0.02$). Laboratory tests including ESR, HLA-B5, HLA-B51 or HLA-B27 and pathergy tests were not different between the two groups (Table 3).

We have found no correlation between the plasma homocysteine level and any organ involvement other than thrombosis. There was also no correlation between the plasma homocysteine level and high ESR, positive pathergy test, HLA-B5 or HLA-B27. The comparisons, done for each subgroup separately with their P values are shown in Table 4.

The mean plasma homocysteine level was significantly lower in HLA-B51 positive BD patients ($11.6 \pm 12.1 \mu\text{Mol/L}$) than in HLA-B51 negative cases ($21.7 \pm 16.3 \mu\text{Mol/L}$) with $P = 0.004$. However, this difference was not significant in those with thrombosis ($20.9 \pm 13.2 \mu\text{Mol/L}$ in HLA-B51 positive patients versus $29.5 \pm 12.7 \mu\text{Mol/L}$ in HLA-B51 negative patients, $P = 0.18$).

Discussion

In our study higher levels of homocysteine in Iranian BD patients were found compared to healthy controls. This is in concordance with several reports on Turkish patients with BD.^{19–22,24–26,28} An increased plasma homocysteine level was shown to be associated with a tendency to induce thrombosis both by endothelial damage and activation of the clotting cascade.^{29,30} This role of the contribution of elevated serum homocysteine levels to thrombosis in BD was suggested by Ates et al. in 45 Turkish BD patients (16 with vascular involvement) with significantly higher levels in patients with vascular involvement than in

those with mucocutaneous involvement ($P < 0.01$).²² No association was found in 79 BD patients from eastern Spain (23 with thrombosis) by Ricart et al.³¹ Iranian BD patients in our study, as with the Turkish cases, showed significantly higher homocysteine levels in those with thrombosis, while homocysteine levels were comparable in healthy controls and BD patients without thrombosis.

Although higher levels seen in male patients might explain the higher incidence of thrombosis in men, no sex difference in homocysteine levels was shown in those with thrombosis in our study. This was the same as previously seen in Turkish patients in whom male sex was not independently associated with thrombosis in multiple regression analysis.²²

As expected, there was no correlation between the plasma homocysteine level and any organ involvement other than thrombosis (Table 4). The lower frequency of ocular lesions in BD patients with thrombosis was in contradiction with our previous reports on vascular involvement in BD.² Sarican et al. assumed homocysteine to be a risk factor and a marker for activation of BD.²⁸ We have not checked this possible relation with disease activity in our patients.

Looking for a genetic basis for the hyperhomocysteinemia and thrombosis in BD, Canataroglu et al. found a similar distribution for the methylenetetrahydrofolate reductase (MTHFR) gene C677T mutation in BD patients and controls despite higher plasma homocysteine levels in BD patients with thrombosis.²¹ While den Heijer et al. showed a 20% increased risk for vascular thrombosis with MTHFR gene C677T mutation in BD patients.³² We are currently checking this mutation in our patients and data will be presented later.

We have noticed a possible negative association between HLA-B51 and homocysteine levels in this study. To the best of our knowledge, this is the first study showing a negative correlation between HLA-B51 and plasma homocysteine levels. We have determined HLA-B51 only in less than one-third of BD patients and none of the controls in this study. Due to this bias and, as there was no correlation between HLA-B51 and thrombosis both in this study and previous reports in Iranian patients with BD,³³ this has suggested that the potential negative contribution of HLA-B51 to induce thrombosis in BD patients needs further evaluation. Further studies are needed both in the normal population and in a higher number of BD

patients.

In conclusion, plasma homocysteine levels may be an independent risk factor for venous or arterial thrombosis in Iranian patients with BD. Further prospective studies are needed before we can recommend plasma homocysteine measurement in new BD cases in order to determine who are likely to develop major thrombotic events.

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References

- Lie JT. Vascular involvement in Behcet's disease: arterial and venous and vessels of all sizes. *J Rheumatol.* 1992; **19**: 341 – 343.
- Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. *N Engl J Med.* 1999; **341**: 1284 – 1291.
- Leiba M, Sidi Y, Gur H, Leiba A, Ehrenfeld M. Behcet's disease and thrombophilia. *Ann Rheum Dis.* 2001; **60**: 1081 – 1085.
- Shahram F, Nadji A, Jamshidi A, Davatchi F. Large vessel involvement in Behcet's disease. *Acta Med Iran.* 2004; **42**: 158 – 162.
- Koc Y, Gullu I, Akpec G, Akpolat T, Kansu E, Kiraz S, et al. Vascular involvement in Behcet's disease. *J Rheumatol.* 1992; **19**: 402 – 410.
- Gul A, Ozbec U, Ozturk C, Inanc M, Konice M, Ozcelik T, et al. Coagulation factor V gene mutation increases the risk of venous thrombosis in Behcet's disease. *Br J Rheumatol.* 1996; **35**: 1178 – 1180.
- Kural-Seyahi E, Fresko I, Seyahi N, Ozyazgan Y, Mat C, Hamuryudan V, et al. The long-term mortality and morbidity of Behcet's syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. *Medicine (Baltimore).* 2003; **82**: 60 – 76.
- Calamia K, Schirmer M, Melikoglu M. Major vessel involvement in Behcet's disease. *Curr Opin Rheumatol.* 2005; **17**: 1 – 8.
- Kosar A, Haznedaroglu I, Buyukasik Y, Kirazly S, Dundar S. Activated protein C resistance in Behcet's disease. *Rheumatol Int.* 1988; **17**: 249 – 250.
- Nalcaci M, Pekcelen Y. Antithrombin III, protein C, and protein S plasma levels in patients with Behcet's disease. *J Med Res.* 1998; **26**: 206 – 208.
- Navarro S, Ricart J, Medina P, Vaya A, Villa P, Todoli J, et al. Activated protein C level in Behcet's disease and risk of venous thrombosis. *Br J Haematol.* 2004; **126**: 550 – 556.
- Kwon S, Lim M, Park S, Moon Y, Park W. Decreased protein S activity is related to the disease activity of Behcet's disease. *Rheumatol Int.* 2006; **27**: 39 – 43.
- Al-Daalan A, Al-Ballaa S, Al-Janadi M, Bholega S, Bhabri S. Association of anticardiolipin antibodies with vascular thrombosis and neurological manifestation of Behcet's disease. *Clin Rheumatol.* 1993; **12**: 28 – 30.
- Priori R, Conti F, Pittoni V, Garofalo T, Sorice M, Valesini G. Is there a role for antiphospholipid binding protein antibodies in the pathogenesis of thrombosis in Behcet's disease? *Thromb Haemost.* 2000; **83**: 173 – 174.
- Tokay S, Direskeneli H, Yurdakul S, Akoglu T. Anticardiolipin antibodies in Behcet's disease: a reassessment. *Rheumatology (Oxford).* 2001; **40**: 192 – 195.
- Guerey A, Gurler A, Oner A, Mesci L, Kirazli S. Thrombomodulin levels in Behcet's disease with and without factor V Leiden mutation. *Clin Rheumatol.* 1998; **17**: 186 – 188.
- Gurgey A, Balta G, Boyvat A. Factor V Leiden mutation and PAI-1 gene 4G/5G genotype in thrombotic patients with Behcet's disease. *Blood Coagul Fibrinolysis.* 2003; **14**: 121 – 124.
- Leiba M, Seligsohn U, Sidi Y, Harats D, Sela B, Griffin J, et al. Thrombophilic factors are not the leading cause of thrombosis in Behcet's disease. *Ann Rheum Dis.* 2004; **63**: 1445 – 1449.
- Altinbas A, Aytemur K, Tokgozoglu L, Ozturk M, Kosar A, Haznedaroglu I, et al. Hyperhomocysteinemia and activated protein C resistance in Behcet's disease. *J Intern Med.* 2000; **248**: 267 – 269.
- Aksu K, Turgan N, Oksel F, Keser G, Ozmen D, Kitapcioglu G, et al. Hyperhomocysteinemia in Behcet's disease. *Rheumatology (Oxford).* 2001; **40**: 687 – 690.
- Canataroglu A, Tanriverdi K, Inal T, Seydaoglu G, Arslan D, Ozbek S, et al. Methylenetetrahydrofolate reductase gene C677T mutation and plasma homocysteine level in Behcet's disease. *Rheumatol Int.* 2003; **23**: 236 – 240.
- Ates A, Aydintug O, Olmez U, Duzgun N, Duman M. Serum homocysteine level is higher in Behcet's disease with vascular involvement. *Rheumatol Int.* 2005; **25**: 42 – 44.
- Hankey G, Eikelboom J. Homocysteine and vascular disease. *Lancet.* 1999; **354**: 407 – 413.
- Mungan A, Can M, Acikgoz S, Esturk E, Altinyazar C. Lipid peroxidation and homocysteine levels in Behcet's disease. *Clin Chem Lab Med.* 2006; **44**:

- 1115 – 1118.
25. Ozkan Y, Yardim-Akaydin S, Sepici A, Engin B, Sepici V, Simsek B. Assessment of homocysteine, neopterin, and nitric oxide levels in Behcet's disease. *Clin Chem Lab Med.* 2007; **45**: 73 – 77.
 26. Ozdemir R, Barutcu I, Sezgin A, Acikgoz N, Ermis N, Esen A, et al. Vascular endothelial function and plasma homocysteine levels in Behcet's disease. *Am J Cardiol.* 2004; **94**: 522 – 525.
 27. International Study Group for Behcet's disease. Criteria for diagnosis of Behcet's disease. *Lancet.* 1990; **335**: 1078 – 1080.
 28. Sarican T, Ayabakan H, Turkmen S, Kalaslioglu V, Baran F, Yenice N. Homocysteine: an activity marker in Behcet's disease? *J Dermatol Sci.* 1990; **45**: 121 – 126.
 29. Woo K, Chook P, Lolin Y, Cheung A, Chan L, Sun Y, et al. Hyperhomocysteinemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation.* 1997; **96**: 2542 – 2544.
 30. D'Angelo A, Selhub J. Homocysteine and thrombotic disease. *Blood.* 1997; **90**: 1 – 11.
 31. Ricart J, Vaya A, Todoli J, Calvo J, Villa P, Estelles A, et al. Thrombophilic risk factors and homocysteine level in Behcet's disease in eastern Spain and their association with thrombotic events. *Thromb Haemost.* 2006; **95**: 618 – 624.
 32. den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. *J Thromb Haemost.* 2005; **3**: 292 – 299.
 33. Shahram F, Davatchi F, Nadji A, Jamshidi A, Bahar K, Akbarian M, et al. Association of HLA-B51 with clinical expression of Behcet's disease, analysis of 201 Iranian patients. *Adv Exp Med Biol.* 2003; **528**: 147 – 148.



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