



ROLE OF SERUM HOMOCYSTEINE AND VENOUS DOPPLER ULTRASONOGRAPHY IN VENOUS INSUFFICIENCY IN BEHCET'S DISEASE AT ZAGAZIG UNIVERSITY HOSPITALS.

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ABSTRACT

Background: Lower extremity venous thrombosis (LEVT) is the most frequent form of vascular involvement in Behcet's disease. Up to 17% of the mortality in Behcet's disease is reported to be associated with venous involvement such as pulmonary embolism or Budd–Chiari syndrome (BCS). Early recognition and appropriate management of vascular involvement in BD is essential to reduce associated morbidity and mortality. Homocysteine (hcy) may play a role in BD patient with venous affection.

Objectives: To find out the role of homocysteine and venous Doppler ultrasonography in detection of venous insufficiency (VI) in BD patients.

Subjects and Methods: A case control study, 50 patients with Behcet's disease and 50 age and sex matched healthy controls were included. Serum homocysteine (by ng/l) was determined by ELISA. Also measurement of VI by venous Doppler ultrasonography in lower limbs of Behcet's patients and controls.

Results: In this study there was statistically significant difference between serum homocysteine level among BD patients and controls ($p < 0.05$). There was a significant difference between serum homocysteine levels in BD patients with venous versus non venous affections, $p < 0.05$. There was a significant positive correlation between serum hcy levels and VI grades by Doppler US.

Conclusions: Behcet's disease patients with hyperhomocysteinaemia and VI diagnosed by Power Doppler US should be considered as a strong indicator of pathological venous involvement.

Keywords: Behcet's disease, hyperhomocysteinaemia (Hhcy), venous Doppler Ultrasonography, venous insufficiency (VI).

INTRODUCTION

Behcet's disease (BD) is a systemic inflammatory vasculitis of unknown etiology, which presents with recurrent orogenital ulceration, uveitis, and erythema nodosum. Other involvement may characterize the course of the disease: articular, vascular, central nervous system, and gastrointestinal involvements [1].

Behcet's disease is more prevalent in the Mediterranean countries such as Turkey, Iran and Far East including Korea and Japan where the prevalence of HLA B 51 is higher than the general population. [2].

Thrombotic events have been reported in ≈ 10 –40% of patients with BD. Vascular lesions consist of venous and arterial

thrombosis and various types of arterial aneurysms. Although involvement of all types and sizes of blood vessels may occur, in the lower extremity venous thrombosis predominates. Endothelial injury due to vascular inflammation is considered to be an important factor of thrombosis [3].

Homocysteine (hcy) is a sulfur-containing amino acid produced during the metabolism of the essential amino acid methionine in all cells through the normal methylation process. Its sulphhydryl group can cause direct endothelial cytotoxicity, inhibition of glutathione peroxidase and nitric oxide, interference with clotting factor, and oxidation of LDL.[4]

Hyperhomocysteinaemia (Hhcy) may cause lipid peroxidation, impaired vasomotor regulation and prothrombotic surface, therefore lead to vascular endothelial injury and atherothrombogenesis[4-5]. Some studies have shown an association between Hcy levels and endothelial dysfunction in patients with BD[5].

Doppler ultrasound (US) is an important modality for the evaluation of vascular pathology to provide flow information in addition to vascular morphology. The most important advantages are that it is technically easily applicable, cheap and non-invasive [6].

Some studies suggest a high prevalence of venous insufficiency (VI) and some cases of asymptomatic thrombosis in patients with BD [6-7]. Early recognition and appropriate management of vascular involvement in BD is essential to reduce associated morbidity and mortality. [8-9].

In this study we aimed to assess the role of serum homocysteine and venous Doppler Ultrasonography in detection of venous insufficiency (VI) in BD patients.

SUBJECTS AND METHODS

This case control study included 50 patients with Behcet's disease and 50 apparent healthy subjects as control group. BD patients were randomly selected from Rheumatology and Rehabilitation Department, Zagazig University Hospitals, between August 2014 and July 2017. All patients fulfilled the revised international criteria of Behcet disease (ICBD 2010) [10]. Exclusion criteria included patients with autoimmune diseases. Seronegative spondyloarthropathies, renal failure, cardiovascular diseases, liver diseases, cancers, diabetes mellitus and malnutrition. Hyperlipidemic disorders as in alcohol

consumption, obesity, oral contraceptives, metabolic syndrome or hypothyroidism. Blood diseases as leukemia, lymphoma and idiopathic thrombocytopenic purpura and old age >60 years were excluded. Written and verbal consent was taken from all subjects.

All patients were subjected to full history taking, general and locomotor examination. Routine laboratory investigations including CBC, ESR, CRP, liver function test, kidney function test and Lipid profile were done.

Assessment of homocysteine levels in serum samples by specific sandwich enzyme-linked immunosorbent assay (ELISA) kit supplied by Sun Red Company. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Homocysteine (HCY) in samples.

Venous Doppler Ultrasonography:

This study was performed with 6.2 to 11 MHz linear multiband probe. All patients were evaluated by a single radiology doctor. Venous vessels of each side at the lower extremities were examined while the subjects were in supine position.

Almost all veins of both lower limbs (the common femoral vein, femoral vein, popliteal vein, anterior tibial vein, posterior tibial vein, and long saphenous vein and short saphenous vein) were examined by Doppler ultrasonography for detection of venous insufficiency.

When venous insufficiency was identified by Doppler US, a venous insufficiency (VI) grading system was used (CEAP) severity score [7] *table 1*. The reverse flow <1 second was accepted as physiological and a reverse flow ≥ 1 second with valsalva maneuver as abnormal [11].

Table 1 Definitions of CEAP severity grades[7]:

Grade	Definition
0	Normal valve competence; no reflux is demonstrated beyond the upper limit of the femoral vein
1	Minimal incompetence; reflux exists beyond the upper most valve in the femoral vein, but not beyond the proximal aspect of the thigh
2	Mild incompetence; reflux occurs in the femoral vein to the level of the knee
3	Moderate incompetence; reflux occurs below the knee
4	Severe incompetence; reflux occurs into the pair calf veins to the level of the ankle

(CEAP) = *Clinical, Etiologic, Anatomic, Pathophysiologic*

Statistical analysis: The collected data were analyzed using the statistical package for social sciences version 13 (SPSS Inc., Chicago, USA).

RESULTS

The BD patients were 13 females and 37 males, their ages ranged from 20-57years, with a mean of 34.8 ± 8.5 years. The control group included 18 females and 32 males.

Their ages ranged from 19-54years with a mean of 31.9 ± 8.1 years. There was no statistically significant difference between patients and controls as regards age or gender. In BD patients the most frequent clinical variables were oral ulcers (88%) and the least frequent manifestation was GIT affection (2%) while vascular (venous) affection was representing (36%).

Table (2): Levels of serum homocystiene in the studied groups:

Items	Studied groups		MW	* <i>p</i>
	Behcet patients (n=50)	Controls (n=50)		
Serum homocystiene				
Mean $\bar{x} \pm SD$	8.8 \pm 6.7	4 \pm 2.8	32.8	<0.05 (S)
Range (ng/L)	(2.8-30)	(1.4-14)		
Median(ng/L)	5.85	3.3		

**Mann-Whitney. P < 0.05 significant*

There was significant difference between serum homocystiene levels in control subject versus BD patients ($p < 0.05$). Table (2).

Table (3): Serum homocystiene levels in patients with and without vascular venous affection:

Serum homocystiene	Behcet patients with venous affection (n=18)	Behcet patients without venous affection (n=32)	t	** <i>p</i>
Mean $\bar{x} \pm SD$	(15.7 \pm 6.8)	(4.9 \pm 1.1)	8.8	<0.05 (s)
Range (ng/L)	(7.6-30)	(2.8-7.2)		

There was a significant difference between serum homocystiene levels in BD patients with and without venous affections, ($p < 0.05$). Table (3).

Table (4): Frequency of grades of venous insufficiency among studied groups by doppler Ultrasonography:

	0 n (%)	1 n (%)	2 n (%)	3 n (%)	4 n (%)	P
	Normal valve competence	Minimal incompetence	Mild incompetence	Moderate incompetence	severe incompetence	
Patients	14 (28)	21 (42)	8 (16)	4 (8)	3 (6)	<0.001
Controls	0	2 (4)	0	0	0	

The most frequent Doppler US CEAP grading was (grade 1) 42% and the least frequent was (grade 4) 6% in BD patients group while among controls 4% were (grade 1) who were obese but without dyslipidemia, diabetes or hypertension. There was a highly significant difference between both groups, $p < 0.001$. Table (4).

Table (5): Serum homocysteine level in relation to grading of venous insufficiency using Ultrasonography in Behcet's patients:

Hcy	CEAP grading					F	P
	0	1	2	3	4		
$\bar{x} \pm SD$	4.1±0.8	5.5±1.7	11.4± 4.4	17.1±6.6	24.5±8.1	31.6	<0.001
Range (ng/L)	(2.8-5.2)	(3.2-12)	(6-19.1)	10.8-28	15.2-30		

There was a highly significant relation between serum homocystiene levels and grades of VI By Doppler US in BD patients, $p < 0.001$.

Table (6): Correlation between serum hcy levels and grades of venous insufficiency by Doppler Ultrasonography Behcet's patients:

	r	P
Serum hcy and (VI)	0.8	p< 0.001

There was a highly statistically significance and strong positive correlation between serum hcy and VI by Doppler US using CEAP severity grades. Table (6) Figure (1).

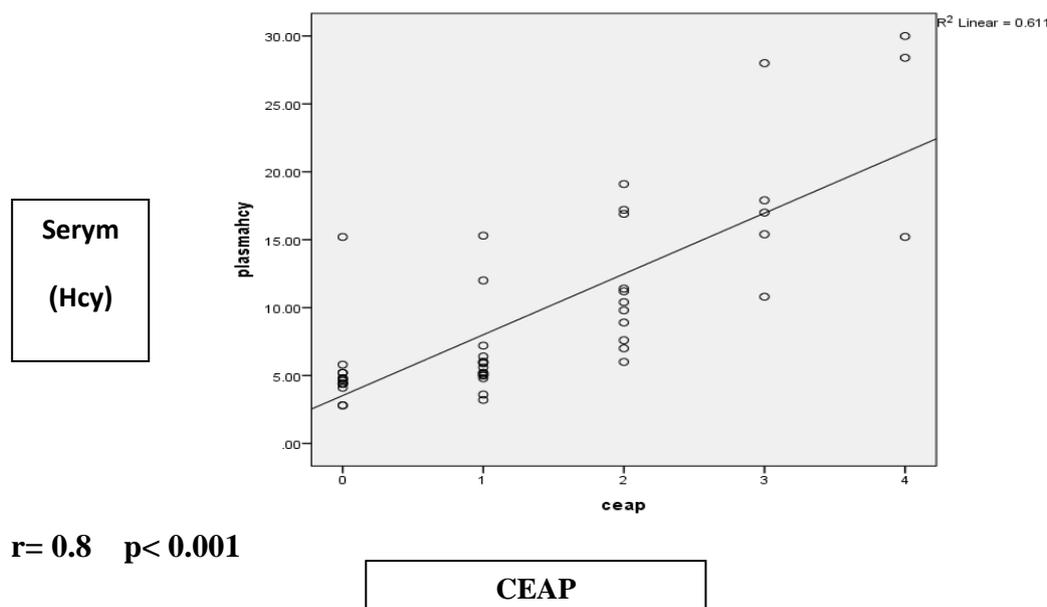


Figure (1):Correlation between serum hcy levels and grades of (VI) by Doppler Ultrasonography in Behcet's patients showing a highly significant positive correlation.

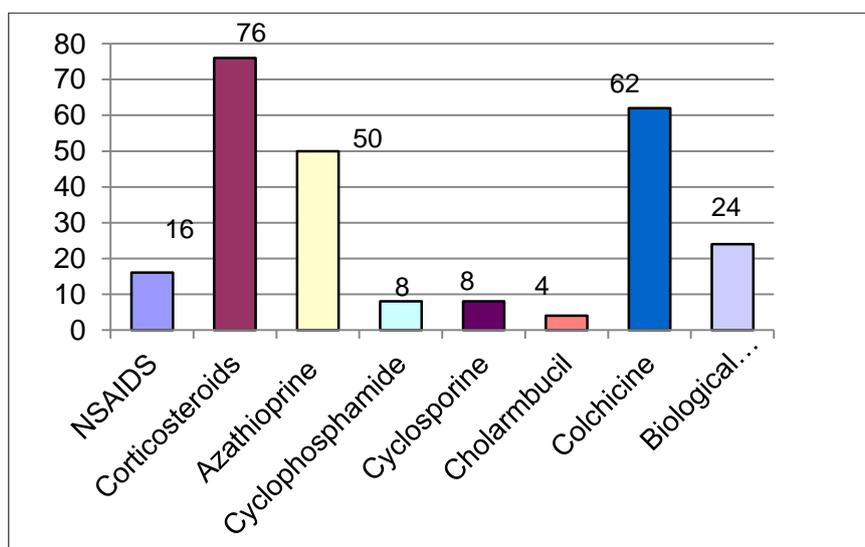


Figure (2): Percentages of Behcet's patients treated with different medications:

The most frequent used medication among BD patients in this study was corticosteroids (76%) followed by colchicine and azathioprine (62%) and (50%) respectively while least used medication was cholarambucil (4%).

DISCUSSION

This research aimed to investigate the relation between serum homocysteine level and vascular affection in BD patients. Also the role of venous Doppler US for both lower limbs veins of BD patients in detection of venous insufficiency.

In this study there was a statistically significant difference between serum homocysteine level in BD patients and controls ($p < 0.05$). That was nearly similar to results of **Ozdemir et al.** [12] and **Wu Y-H, et al.** [13] who found a statistically significant difference of s. Hcy levels between patients versus control ($p < 0.001$).

Our study showed a significant difference between serum homocystiene levels in BD patients with venous non venous affections, $p < 0.05$. **Bahsi et al.** [14], **Ates et al.** [15], **Shahram et al.**[16], **La Regina et al.** [5] and **Durmazlar et al.** [17] also confirmed our results that serum homocysteine was significantly higher in vascular affection in BD patients in comparison with patients without vascular involvement. Although **Shadmanfar et al.** [18] and **Senturk et als** [19] found that there was no statistical difference in hcy levels

between BD patients with and without thrombosis

These results may be explained by that the vascular thrombosis in BD is accepted to be the result of the inflammation of the vessel wall. Hcy generates superoxide and hydrogen peroxide, both of which have been linked to endothelial damage [20]. Hcy-induced vascular problems are thought to be multifactorial, including direct Hcy damage to the endothelium, enhanced lipid peroxidation and increased platelet aggregation by the effects on the coagulation system [17].

In our study there was no significant correlation between serum homocysteine level and CRP ($r = 0.19$, $p > 0.05$) that was in agreement to the study of **Allam et al.** [20], on contrary, **Ozdemir et al.** found a significant correlation between serum homocysteine level and CRP ($r = 0.45$, $P < 0.05$).

In our study there was no significant correlation between serum homocysteine level and ESR ($r = 0.16$, $p > 0.05$) that was in accordance to the study of **Shahram et al.**[16].

US CEAP severity score was used to evaluate the severity of the venous insufficiency by venous Doppler ultrasonography (US) in lower extremities, In our study venous insufficiency findings were detected in 36 of 50 patients (72%) in the BD group but 2 out of 50 (4%) in the healthy control group. **Kisacik et al.**[7]found nearly

similar results of ours (74%); however they found VI in 23% of the healthy control group. While **Alibaz-Oner et al.**[6] found VI in 32.2% in BD patients group and 9.3 % in the healthy control group. Also **Hassan et al.**[21] found VI in (13.6%) BD patients and in (4.5%) control in the form of a reverse of blood flow ≥ 1 sec. This discrepancy may due to difference in sample size and in patients' selection that exclude any BD patients with history of venous affection.

In the current study, Behcet disease group had a significantly higher US CEAP severity scores than healthy controls, $P < 0.001$, these results are in agreement with **Kisacik et al.**[7] and **Alibaz-Oner et al.**[6].

In present study it was found that among BD patients the most frequent Doppler US CEAP grading of VI was (*grade 1*) 42% *Minimal incompetence* (valve reflux exists beyond the uppermost valve in the femoral vein, but not beyond the proximal aspect of the thigh) and the least frequent was (*grade 4*) 6% *Severe incompetence* (valve reflux occurs into the pair calf veins to the level of the ankle) these results could be attributed to the corticosteroid and immunosuppressive medications received by most of the studied patients for vascular BD manifestations and their role in suppressing venous wall inflammation and hence thrombosis.

The results of the present study are in accordance with the study of **Desbois et al.** [22] which deduced that immunosuppressive agents improve prognosis by decreasing the odds of venous thrombosis relapse in BD. Also **Seyahi** (23) concluded that immunosuppressive medications are essential in preventing the attacks and increasing survival of vascular involvement in BD.

The relationship between inflammation and venous thrombosis has recently been highlighted. Inflammation may lead to venous thrombosis by inhibiting both anti-coagulant pathway and fibrinolytic activity. Also endothelial damage and dysfunction have been incriminated in pathogenesis of VI in BD [21]. **Desbois et al.**[22] assessed factors associated with thrombosis relapse and mortality. They mentioned that the use of immunosuppressive

drugs was found to prevent relapse of venous thrombosis and there was a trend towards prevention of relapse with the use of steroids.

In current study there was a highly significant relation between serum homocysteine levels and grades of Doppler US CEAP score in BD patients, ($p < 0.001$). Also there was a statistically significant difference and strong positive correlation between plasma hcy and CEAP grades of VI by Doppler US ($r = 0.8$), To our currently knowledge there is no similar studies.

In this study the commonly used drugs in our patients was corticosteroids, colchicine and azathioprine. Nearly similar results were recorded by **Gheita et al.**[24] where the most commonly used drugs were corticosteroids, colchicine and azathioprine.

Conclusions: Hyperhomocysteinaemia might be an independent risk factor for venous insufficiency in BD. As a non-invasive method, Doppler Ultrasonographic screening of lower extremities may detect early venous insufficiency in BD even in patients without symptomatic or history of thrombosis. Behcet disease patients with both hyperhomocysteinaemia and VI diagnosed by Power Doppler US should be considered as a strong indicator of pathological venous involvement.

REFERENCES

- 1-Hatemi G, Christensen R, Bang D, Bodaghi B, Ferhat C, Fortune F, et al. 2018 update of the EULAR recommendations for the management of Behçet's syndrome. *Ann Rheum Dis.* 2018; 77 (6): 1–11.
- 2- Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis.* 2001; 60(11):996–1002.
- 3.Nazarinia M. A., Aflaki E., Habibaghahi Z., Rajaei A. and Omrani G. R. Serum homocysteine level and vascular thrombosis in patients with Behçet disease. *APLAR Journal of Rheumatology* 2007; 10: 178–81.
- 4.Sarican T, Ayabakan H., Turkmen S., Kalaslioglu V., Baran F., and Yenice N. "Homocysteine: an activity marker in Behçet's disease?" *J Dermatol Sci.* 2007; 45(2):121–6.
- 5-La Regina M, Orlandini F, Prisco D and Dentali F: Homocysteine in vascular Behçet disease: a meta-analysis. *Arterioscler Thromb Vasc Biol.* 2010; 30(10):2067-74.

- 6-Alibaz-Oner F, Karatay E, Akpınar IN, Ergun Tandireskeneli.H.Evaluation of asymptomatic venous disease by venous Doppler ultrasonography in patients with Behcet's disease without overt thrombosis. *ClinRheumatol.* 2014; 33:277–80.
- 7.Kisacik B, Oren C, Kasifoglu T.,Sedat Y,YilmazO,SimsekI et al. Investigation of the veins in patients with Behçet's disease with no known vascular event by Doppler ultrasonography. *Rheumatol Int.* 2012; 32(2):303–6.
- 8- Kaklamanisn PG andCalamia KT. Behçet's disease: an update on pathogenesis,diagnosis and management of vascular involvement. *Rheumatol Rep.* 2010; 2:e2.
- 9-Fei Y, Li X, Lin S, Song X, Wu Q, Zhu Y, et al. Major vascular involvement inBehçet's disease: a retrospective study of 796 patients. *ClinRheumatol.*2013;32:845–52.
- 10-Davatchi F, Assaad-Khalil S, Calamia KT.,Crook, J. E., SadeghiA., Schirmer, M. et al: The International Criteria for Behcet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. *J EurAcadDermatolVenereol.* 2014; 28(3): 338-47.
- 11-Labropoulos N, Tiongson J, PryorL,Tassiopoulos A, Kang S, Mansour A. et al. Definition of venous reflux in lower-extremityveins.*J Vasc Surg.* 2003; 38:793-8.
- 12-Ozdemir R., Yagmur J., Acikgoz N.,CanselM.,Karincaoglu Y., Ermis N., etal.Relationship between serum homocysteine levels and structural-functional carotid arterial abnormalities in inactive Behçet's disease.*KardiologiaPolska.*2018;76, (2): 413–7.
- 13-Wu YH, Yu-Fong C J, Wang YP,Wu YC, Chen HM and Sun A.: Hemoglobin, iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia in Behcet's disease patients with atrophic glossitis. *J Formos Med Assoc.* 2018;117(7):559-65.
- 14- Bahsi M., Ilhan N. and CicekD .: Homocysteine Metabolism and Haemostatic Factor in Behcet's. *Pharma. Chem. J.* 2017; 4 (2):72-9.
- 15- Ates A, Aydintug O, Olmez U, Duzgun NandDuman M: Serum homocysteine level is higher in Behcet's disease with vascular involvement. *Rheumatol Int.* 2005; 25:42–4.
- 16- Shahram F, Faridar A, Hamedani MG Nadji A, Naderi N, MojaradShafiee N,et al. Plasma homocysteine level in patients with Behcet's disease with or without thrombosis. *Arch Iran Med.* 2010; 13: 476–81.
- 17- Durmazlar SPK D., Akgul A and Eskioglu F.Homocysteine may involve in the pathogenesis of Behcet's disease by inducing inflammation. *MediatInflamm.* 2008; (7)1-9.
- 18- Shadmanfar S., Shahram F., Nadji A., Akhlaghi M., Faezi S.,Sadeghi-abdollahi B.,et al.The relationship between plasma homocysteine level and HLA-B51 in patients with Behcet's disease: a case-control study. *Int J Rheum Dis.* 2014; 17: 466–70.
- 19- Senturk O, Ozgur O, Hulagu S,Canturk NZ, Celebi A andKarakaya T.Effect of Helicobacter pylori infection on deep vein thrombosis seen in patients with Behcet's disease. *East African Med J.* 2006; 83:49 –51.
- 20- Allam A., Ammar H. and Radwan A. : Serum homocysteine level and eye involvement in Egyptian patients with Behcet's disease. *Egypt Rheumatol.* 2014; (36), 29–34.
- 21-Hassan S.Z., Gheith RE , Baz AA.andAfifi Z.: Evaluation of asymptomatic venous disease by Doppler ultrasonography in Behçet's disease patients. *Egypt Rheumatol.* 2017; 39:165–70.
- 22-Desbois AC, Wechsler B, Resche-Rigon M., Piette JC, Le ThiHuong D,Amoura Z, et al. Immunosuppressants reduce venous thrombosis relapse in Behcet's disease. *Arth Rheum.*2012; 64(8):2753–60.
- 23-Seyahi E.: Behçet's disease: how to diagnose and treat vascular involvement. *Best Pract Res ClinRheumatol.* 2016; 30(2):279–95.
- 24- Gheita TA, Samir H and Hussein H. Anti-annexin V antibodies in neuro-Behcet patients: clinical significance and relation to disease activity. *Int J Rheum Dis* 2012; 15(5):e124–6.

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