B Vitamin Supplementation Reduced Serum Homocysteine and Interleukin-6 Levels in Patients with Behcet's Disease with Acute Venous Thrombosis: A Prospective Controlled Study

Akut Venöz Trombozlu Behçet Hastalarında B Vitamin Desteği ile Serum Homosistein ve İnterlökin-6 Düzeylerinin Düşürülmesi: Prospektif Kontrollü Çalışma

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ABSTRACT Objective: Some previous studies suggested that hyperhomocysteinemia and pro-inflammatory cytokines were risk indicators of venous thrombosis in Behcet's disease (BD). However, a prospective trial investigating the effect of homocysteine (Hcy) reduction on interleukin-6 (IL-6) level, a marker of inflammation, has not been performed previously. Our aim was to evaluate the effect of Hcy lowering therapy on serum levels of IL-6 in patients with BD with venous thrombosis. **Material and Methods:** Thirty patients with BD with acute venous thrombosis of the leg and 30 healthy subjects were included in this study. Serum levels of Hcy and IL-6 were evaluated in both groups. Later on, 15 patients were randomized to receive B-vitamin supplements for 8 weeks and serum levels of Hcy and IL-6 were reevaluated. Remaining 15 patients made the control group serum levels of IL-6 were reevaluated after 8 weeks. **Results:** Serum Hcy and IL-6 levels were found significantly elevated in patients with BD with venous thrombosis when compared with healthy controls (p= 0.00). Hcy was found to have high correlation with serum IL-6 level (r= 0.802, p= 0.00). After vitamin supplementation for 8 weeks, Hcy levels of patients were found significantly decreased (p= 0.001). IL-6 levels were found significantly decreased in patients receiving vitamin supplements when compared with patients who were not receiving vitamin supplements (p= 0.00), suggested that reducing Hcy reduced IL-6 levels. **Conclusion:** Supplementation with folic acid, vitamin B6 and vitamin B12 was effective in normalizing serum homocysteine concentration and reducing IL-6 levels in patients with BD with venous thrombosis.

Key Words: Behcet's syndrome, venous thrombosis

ÖZET Amaç: Daha önceki bazı çalışmalar Behçet hastalığı (BH)'nda hiper homosisteinemi ve proinflamatuvar sitokinlerin venöz trombozun risk belirteçleri olduğunu öne sürdü. Buna rağmen, homosistein (Hcy) azaltılmasının, inflamasyonun bir belirleyicisi olan interlökin-6 (IL-6) üzerine etkisini araştıran prospektif bir çalışma daha önce yapılmadı. Bizim amacımız venöz trombozlu BH'de Hcy azaltan tedavinin serum IL-6 düzeylerine etkisini değerlendirmekti. **Gereç ve Yöntemler:** Bu çalışmaya akut bacak venöz trombozu olan 30 BH ve 30 sağlıklı birey dahil edildi. Serum Hcy ve IL-6 düzeyleri her iki grupta değerlendirildi. Daha sonra 15 hasta, 8 hafta süresince B-vitamin takviyesi almak üzere rastgele seçilerek serum Hcy ve IL-6 düzeyleri tekrar değerlendirildi. Kalan 15 hasta kontrol grubunu oluşturdu ve 8 hafta sonra serum IL-6 düzeyleri tekrar değerlendirildi. **Bulgular:** Sağlıklı kontrollerle karşılaştırıldığında, venöz trombozlu BH'de serum Hcy ve IL-6 düzeyleri belirgin olarak yüksek bulundu (p= 0.00). Hcy'nin serum IL-6 düzeyi ile yüksek korelasyon gösterdiği saptandı (r= 0.802, p= 0.00). Sekiz hafta vitamin desteğinden sonra hastaların Hcy düzeylerinin belirgin olarak düştüğü bulundu (p= 0.00). Vitamin desteği alan hastalarda IL-6 düzeylerinin vitamin desteği almayan hastalarla karşılaştırıldığında belirgin olarak düştüğü bulundu (p= 0.00), bu bulgular Hcy azaltılmasının IL-6'yı azalttığını düşündürttü. **Sonuçlar:** Folik asit, vitamin B6 ve vitamin B12 ile destek tedavisi, venöz trombozlu BH'de serum Hcy konsantrasyonunu normalize etmek ve IL-6 düzeylerini düşürmekte etkiliydi.

Anahtar Kelimeler: Behçet sendromu, venöz tromboz

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Behcet's disease (BD) is a vasculitis, characterized by involvement of both arteries and veins and vessels of all sizes.[1] Thrombosis of superficial and deep vein is more frequent than arterial aneurism and thrombotic occlusions.[2,3] Deep vein thrombosis is seen in about one-fifth of Turkish patients with BD.[2]

Hcy is an intermediary sulphydryl-containing aminoacid formed during the conversion of methionine to cysteine. Its sulphydryl group can cause direct endothelial cytotoxicity.[4] High levels of Hcy cause lipid peroxidation, impaired vasomotor regulation, prothrombic surface, and therefore atherothrombogenesis.[5] Several reports show that Hcy promotes thrombosis by disturbing the procoagulant-anticoagulant balance.[6,7] Some studies have shown that hyperhomocysteinemia might be assumed to be an independent and correctable risk factor for thrombosis in BD.[8-13] Hcy is thought to induce proinflammatory cytokines.[14,15] Vitamin B6, vitamin B12 and folic acid supplementation can decrease homocysteine levels even in people with normal serum vitamin concentrations.[16] Combined vitamin supplementation was reported to reduce hcy level effectively within 8 weeks.[17]

Pro-inflammatory cytokines such as IL-1, tumor necrosis factor (TNF) and IL-6, play a key role in tissue factor expression on monocytes and on endothelial cells and contribute significantly to regulation of hemostatic balance in physiologic and pathologic conditions.[18] Increased spontaneous secretion of TNF-α, IL-6 and IL-8 in monocyte cultures obtained from BD patients have been shown.[19] Available experimental and clinical evidence suggests that IL-6 is mainly involved in activation of coagulation.[18]

Based on this background, our aim was to evaluate the serum levels of Hcy and IL-6 in patients with BD with venous thrombosis of the leg and the results were controlled with healthy individuals. In addition, we evaluated the effect of Hcy- lowering therapy, by B-vitamin supplementation, on serum levels of IL-6 in patients with BD with venous thrombosis.

To the best of our knowledge, our study is the first prospective trial that investigated the effect of homocysteine reduction on IL-6 level, a marker of inflammation, in patients with BD with venous thrombosis.

MATERIAL AND METHODS

SUBJECTS

Thirty patients with deep vein thrombosis with or without superficial thrombophlebitis of the leg (20 male, 10 female; mean age of 38.87 ± 7.16) fulfilling the International Study Group Criteria for the diagnosis of BD and 30 healthy control subjects (21 male, 9 female; mean age of 37.18 ± 6.59) were included in this study.[20] Patients who had concomitant arterial involvement were not included in the study. Patients with BD were selected from patients who were being followed up by the departments of dermatology and cardiovascular surgery. Patients who had other type of illness such as autoimmune disease that could affect the cytokine levels or patients taking any medication affecting the immune system or vitamin supplements in the previous 6 months and patients with hyperlipidemia, chronic hepatitis, renal failure, severe psoriasis, pernicious anemia, and chronic alcoholism which might affect Hcy levels were excluded from the study. The study was approved by the local ethics institute. Informed consent was obtained from all subjects.

STUDY DESIGN

We evaluated the serum levels of Hcy and IL-6 in patients with BD with acute deep vein thrombosis with or without superficial thrombophlebitis of the leg and the results were controlled with healthy individuals.

In the second step of our study, half of the patients (n= 15) were randomized to receive B-vitamin supplements (folic acid 5 mg, cobalamin 0.4 mg and pyridoxine 25 mg), daily for 8 weeks. Eight weeks after, serum levels of Hcy and IL-6 were reevaluated. Remaining patients (n= 15) made the control group and 8 weeks after, serum levels of IL-6 were reevaluated in this group.

VASCULAR EXAMINATION

Venous thrombosis was defined as present when confirmed by Doppler ultrasonography, contrast venography, impedance plethysmography or compression ultrasonography. Superficial thrombophlebitis was confirmed by clinical parameter (palpable elongated subcutaneous lumps along the axis of superficial veins, tenderness, swelling and redness).

SERUM HOMOCYSTEINE ANALYSIS

Serum homocysteine levels in the study groups were determined by fluorescence polarization immunoassay (FPIA) technique (Axis Biochemicals, ASA, Oslo, Norway). FPIA was run on an IMx analyzer (Abbott, USA). The 95% confidence interval of plasma Hcy level suggested by the manufacturer for healthy individuals is 4.45-12.42 μmol/L. 5 cc blood samples were drawn using a 25 gauge needle from a peripheral vein, avoiding haemolysis in the morning hours after an overnight fasting and 30 minutes of supine rest and collected into ethylenediamine tetraacetate (EDTA) containing tubes. Samples were centrifuged at 1000 xg for 10 minutes. The serum was separated in aliquots and immediately frozen and stored at 60°C within 60 minutes until use.

DETECTION OF SERUM IL-6 BY ENZYME-LINKED IMMUNOSORBANT ASSAY

The IL-6 level was measured with an enzyme-linked immunosorbant assay (ELISA) using an Human IL-6 Bio Source International Immunoassay kit (Nivelles, Belgium) with a detectable level set at 2.5 pg/mL. 5 cc venous blood samples were drawn from patients and healthy volunteers. The serum was separated by centrifugation at 1000 xg for 10 min and stored at -70 until use.

STATISTICAL ANALYSIS

Results were analysed using SPSS for Windows® VER 11.5 (SPSS Inc., Chicago, IL, USA). Variants were evaluated by one sample Kolmogorov-Smirnov test for compability with normal distribution. As the data did not fit normal distribution, the nonparametric Mann-Whitney U test was used for between-group comparisons as indicated. The relationship between variables was evaluated by Spearman correlation. Levels of significance set at 0.05. Data are presented as median values and their individual ranges (in parenthesis).

RESULTS

There was no statistically significant difference in the sex (p= 0.921) and age (p= 0.183) of the groups. Serum Hcy levels were found significantly elevated in patients with BD with venous thrombosis when compared with healthy controls with a median of 18.2 (15-28) and 8.1 (5-12.21) respectively (p= 0.00). Serum IL-6 levels were found significantly elevated in patients with BD with venous thrombosis when compared with healthy controls with a median of 38 (28-227) and 2.5 (2.5-27) respectively (p= 0.00) (Table 1). Hcy was found to have high correlation with serum IL-6 levels (r= 0.802, p= 0.00) (Table 2).

After B-vitamin supplementation for 8 weeks, Hcy levels of patients were found significantly decreased when compared with pretreatment values with a median of 12.4 (8.3-17.8) and 18.03 (15-24) respectively (p= 0.001) (Table 2).

IL-6 levels were found significantly decreased in patients receiving B-vitamin supplements when compared to patients who were not receiving B-vitamin supplements with a median of 17 (9-28) and 36 (26-56) respectively (p= 0.00), suggesting that reducing Hcy reduced IL-6 levels.

DISCUSSION

Venous thrombosis appeared to be the major vascular involvement reported in 7 to 33% of cases with BD with a male predominance, and representing 85 to 93% of vasculo-BD.[21] Hyperhomocysteine-

TABLE 1: Comparision of serum homocysteine and IL-6 levels between groups.

	Patients with venous thrombosis (n= 30)	Healthy controls (n= 30)	p value
Hcy levels (μmol/L)	18.2 (15-28)	8.1 (5-12.21)	0.00*
IL-6 levels (pg/mL)	38 (28-227)	2.5 (2.5-27)	0.00*

* Significantly different by Mann Withney U test (p< 0.05)
Data are presented as median values and their individual ranges within parentheses.

TABLE 2: The relationship between serum homocysteine and IL-6 levels.

	Spearman correlation coefficient	p value
Hcy and IL-6 levels	r= 0.802	0.00*

TABLE 3: Comparision of serum homocysteine levels in patients before and after treatment with B-vitamin supplements.

	Patients n= 15		
	Before	After	p value
Hcy levels	18.03 (15-24)	12.4 (8.3-17.8)	0.001*

* Significantly different by Mann Withney U test (p<0.05)
Data are presented as median values and their individual ranges within parentheses.

TABLE 4: Comparision of serum IL-6 levels in patients receiving B-vitamin supplements (Hcy reduced group) with patients who were not receiving B-vitamin supplements.

	Patients receiving vitamin supplements (n= 15)	Patients not receiving vitamin supplements (n= 15)	p value
IL-6 levels (pg/mL)	17 (9-28)	36 (26-56)	0.00*

* Significantly different by Mann Withney U test (p<0.05)
Data are presented as median values and their individual ranges within parentheses.

mia is independently associated with the development of coronary, cerebral and peripheral vascular disease and deep-vein thrombosis in the general population.[22] The main factor responsible for the increased frequency of thrombosis in BD is thought to be endothelial dysfunction caused by vascular inflammation.[8,9] The association between Hcy levels and endothelial dysfunction and its correlation to the degree of endothelial damage have been shown in patients with BD.[23] Some studies have shown that hyperhomocysteinemia might be assumed an independent and correctable risk factor for thrombosis in BD.[8-13] Hcy is thought to induce proinflammatory cytokines.[14,15] In patients with BD, increased spontaneous secretion of cytokines involved in the inflammation such as TNF-α, IL-6 and IL-8 was shown.[24,25] Indeed we recently showed that Hcy levels were elevated in the active stage of BD and correlated with the number of active clinical manifestations increased. We showed a positive correlation between serum Hcy and inflammatory markers; TNF- α, CRP and ESR.[15] As we previously showed that serum hcy levels were elevated in the presence of vascular involvement, activity of the disease were not assessed in this study.[15] Consistent with previous reports in this study we found elevated serum Hcy levels in patients with BD with venous thrombosis when compared with healthy controls and Hcy was found to have high correlation with serum IL-6 levels. In addition, our study showed that reducing Hcy by B-vitamin supplementation reduced IL-6 levels, confirming that Hcy associated with IL-6, a proinflammatory cytokine. We excluded the effect of the result of the decrease of the thrombotic process on serum levels of IL-6 by reevaluating the serum levels of IL-6 after 8 weeks in the control group which consisted of patients that did not receive vitamin supplementation.

Hcy is thought to promote the clotting cascade via several actions including inactivation of protein C, activation of coagulation factor V, increased vascular smooth muscle cell proliferation, and inhibition of thrombomodulin.[9] In vasculitis such as BD, endothelial-leukocyte interaction is an important part of the inflammation. Hcy has been reported to increase endothelial-leukocyte interaction.[26,27] The increase in Hcy concentration in patients at risk for vascular disease is expressed as odds ratio and for venous thrombosis, this odds ratio is approximately 1.6.[28] In a study, a change of 1 μmol/L in Hcy concentration was found to correspond to a risk ratio of 1.01.[28] A study reported that 5 μmol/L increase of Hcy was associated with a 60% and 27% increased risk of venous thrombosis in retrospective and prospective studies, respectively.[29] Additionally, increase of Hcy was found related with stasis dermatitis and ulcer.[30] There is accumulating evidence for inflammation markers, such as IL-6, as a result of thrombosis.[31] As, available experimental and clinical evidence suggests that IL-6 is mainly involved in activation of coagu-

lation, reducing IL-6 levels might be useful in thrombotic events.[18]

CONCLUSION

In our study, we found elevated serum Hcy and IL-6 levels in patients with BD with venous thrombosis when compared with healthy controls. Our study showed that Hcy had a high correlation with serum levels of IL-6, a cytokine mainly involves in activation of coagulation. These results add further evidence for the contention that hyperhomocysteinemia might be assumed a correctable risk factor for thrombosis in BD and Hcy might induce proinflammatory cytokines. Supplementation with folic acid, vitamin B6 and vitamin B12 was effective in normalizing serum homocysteine concentration and reducing IL-6 levels in patients with BD with venous thrombosis. To investigate the effect of vitamin therapy on the progress and prevention of venous thrombosis in BD, researches involving long-term interventions are needed.

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