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Homocysteine serum level and eye affection in Behçet's disease patients

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Abstract:

Background: Vascular endothelial dysfunction represents the major pathogenesis abnormalities in Behçet's disease (BD). Homocysteine (Hcy), a non-protein-forming amino acid was proposed to be involved in the inflammatory process, and increasing homocysteine concentrations in ischemia may lead to leukocyte recruitment in the affected tissue. **Aim:** to find out the possible relation between homocysteine (HCY) serum level and ocular involvement in Behçet's disease (BD) patients. **Methods:** 30 BD patients as classified by the new International Behçet's Disease Criteria (25 males and 5 females with the age mean 33.83 ± 9.41years) and 20 healthy control subjects (age and sex matched) were included. The patients were divided into two groups: those with BD and eye involvement (n=24) and those without ocular involvement (n= 6). **Results:** The mean serum HCY values in BD patients were substantially greater than in healthy controls (37.87 ± 10.31 and 21.80 ± 5.47 µmol/L, respectively; P <0.001). HCY readings were higher in patients with eye involvement than in patients who did not have eye involvement. (38.91 ± 10.13 and 33.72 ± 10.9 µmol/L, respectively) yet, these differences did not reach the level of statistical significance (P>0.278). **Conclusion:** Serum HCY level is increased in BD. No association was found between HCY levels and eye involvement. Because hyperHCY is a treatable risk factor, measurement and monitoring of HCY levels may be a valuable index in the investigation, management and improving the outcome of patients with BD.

Keywords: Behçet's disease , homocysteine , Eye involvement.

1. Introduction:

Behçet's disease (BD) is a systemic autoimmune inflammatory disorder, recurrent ulceration in orogenital areas, eye involvement, and a variety of lesions on the skin with immune-mediated vasculitis and the creation of arterial aneurysms define this condition [1]. Mucocutaneous lesions with neurologic, cardiac, and gastrointestinal involvement are common clinical presentations of the disease [2]. BD affects people in their third or life's fourth decade, males and females are both affected in the same way. The disease is more common in the historic 'Silk Road' populations'which stretches from Eastern Asia to the Middle East and Mediterranean regions, than Western countries populations [3].

One of the most significant and disabling symptoms of BD is eye affection, which, if neglected, it can result in vision loss and blindness [4]. Homocysteine (HCY) is a precursor to methionine that contains Sulphur, as part of the normal methylation pathway, it is synthesised with all cells. Because HCY increases viscosity of blood, it may increase the risk of thrombus development [5]. HCY appears to be harmful to the endothelium in experiments [6], prothrombotic[7], promotes collagen synthesis[8], and reduces nitric oxide bioavailability. [9] In hypertensive patients with C-reactive protein (CRP) and tumour necrosis factor (TNF) are innate proinflammatory indicators, serum HCY plays a part in the body's body's immune response and is a prediction of TNF [10].

Pathogenic inflammatory mechanisms can induce oxidative stress, which could be an endogenous cause of folate insufficiency, oxidative stress can be induced by pathogenic processes, which could be an endogenous cause of folate deficiency, since this vitamin is associated with the production of HCY [11]. Thus The goal of this study was to study if there was a link between serum HCY levels and ocular affection in BD patients.

2. Patients and Methods:

A case study with a control group performed in thirty patients clinically diagnosed to have BD. They were visiting Beni Suef University Hospital's Rheumatology and Rehabilitation Department's outpatient clinic during the period from October 2019 to June 2020.

2.1 Inclusion criteria

Thirty patients clinically diagnosed to have Behçet's Disease, as classified by the new International Behçet's Disease Criteria (ICBD) [12], There were 25 males and 5 females in the study, with a mean age of (33.83 ± 9.41) years. All of the patients were examined by an expert

ophthalmologist for signs of ocular affection, and then were classified into:

Group 1: patients with ocular manifestations of BD.

Group 2: patients without ocular involvement.

This study employed twenty age and sex matched healthy persons (17 men and 3 females; mean age 35.80 ± 7.94 years) as controls. All participants in the study gave their prior permission, which was accepted by the Ethical Review Committee of Beni-suef University's Faculty of Medicine.

2.2 Exclusion criteria

Diabetes mellitus, malnutrition, cancer, Hyperlipidemia, end stage renal failure and cardiovascular diseases.

The following procedures were carried out on all of the patients:

- Taking the full history
- Clinical evaluation which include

Height and Weight

Examination in general

Skin examination: Examination for oral and genital ulcers.

Chest and cardiac examination

Abdominal examination

Neurological examination

Articular examination: arthralgia, arthritis and joint stability.

Muscle examination

1. Investigations:

- Laboratory tests (liver and kidney functions, complete blood count, ESR, urine analysis).
- Determination of homocysteine levels by ELISA technique were done for all patients and control, with a detection range of 0.78-50 μmol/L, no substantial cross-reactivity or interference was found.

• Statistical interpretation

SPSS (statistical application for social science) was used to examine the data.) on an Operating System as follows;

- Mean, range and SD being used to define the quantitative values.

- Percentages and numbers being used to define the qualitative characteristics.

- In parametric data (SD less than 50% mean), Comparing quantitative variables was done using the t-test.

• Insignificant if the value of P is more than 0.05

• Significant if the value of P is less than 0.05

• highly significant statistically if the value of P is less than 0.01 [13]

3. Results:

- The current study was carried out at the University Hospital of Beni-Suef. Patients included 5 females (16.7%) and 25 males (83.3%), Their ages varied from 18 to 50 years old, with a mean of 33.83 ± 9.41. The control's age ranged from 20 to 50 years with a mean of 35.80 ± 7.94 years. In patients with eye involvement, no statistically significant difference between males and females regarding age was found (p=0.848), but females had significantly longer disease duration (P=0.008) as shown in **Table (1)**

Table (1): Demographic data of patients and control:

Variable (patients)	**Number**	**Percent**
Males	25	83.3%
Females	5	16.7%
Variable (patients)	**Range (Minimum-maximum)**	**Mean± SD**
Age (yrs)	18 - 50	33.83 ± 9.41
Disease duration(yrs)	1 - 15	5.97± 3.93
Variable (control)	**Number**	**Percent**
Males	17	85%
Females	3	15%
Variable (control)	**Range (Minimum-maximum)**	**Mean± SD**
Age(yrs)	20 - 50	35.80 ± 7.94

Variable	**With eye involvement (n=24)**			**Without eye involvement (n=6)**		
	Male	Female	P value	Male	Female	**P value**
Mean Age (years)	33.58±10.44	34.6±10.64	0.848	34.00±5.40	-	**-**
Mean disease duration (years)	5.05±3.10	10.40±5.46	0.008*	5.17±2.79	-	**-**

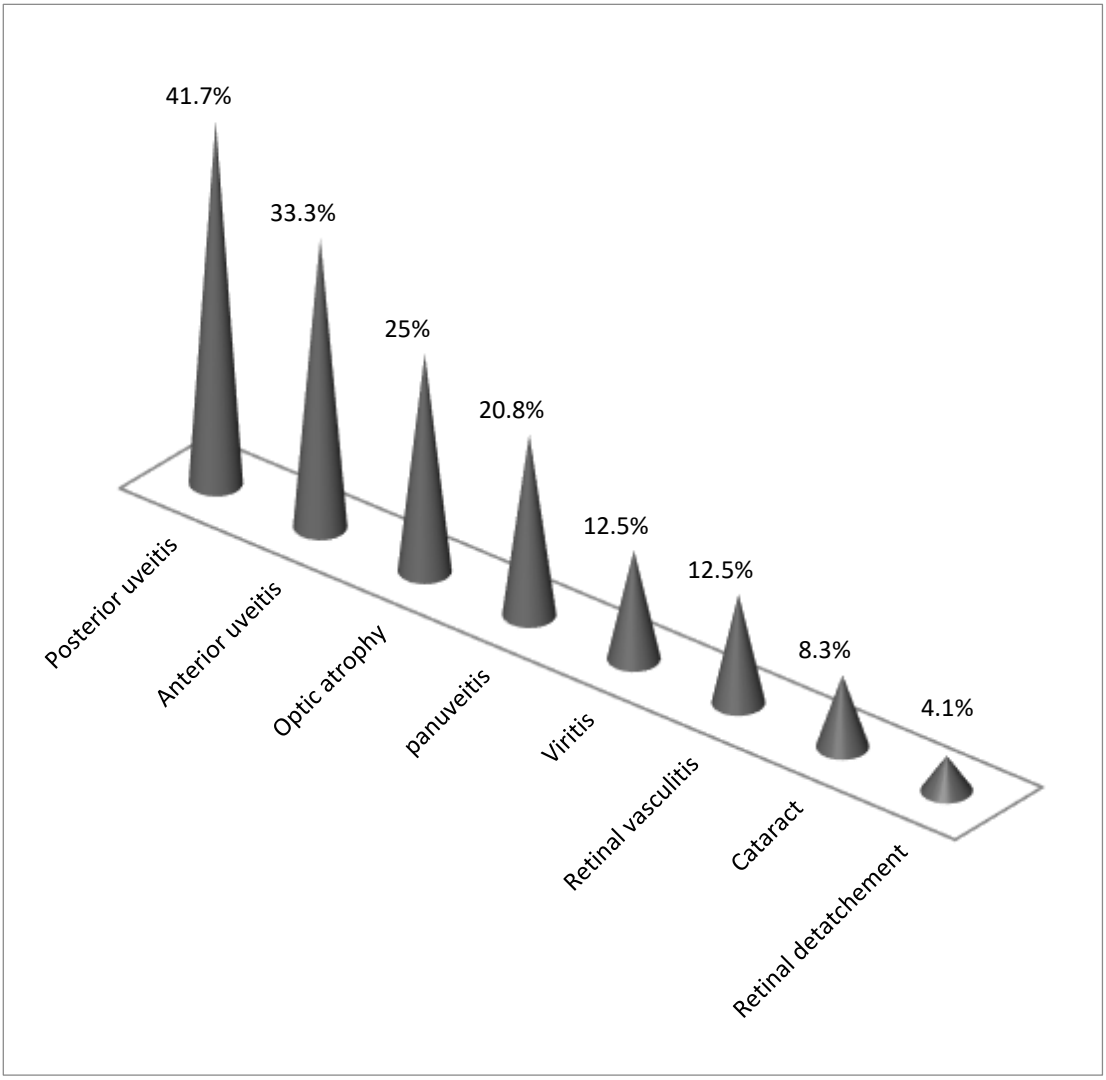
The blood level of HCY was considered to be greater significantly in BD patients (37.87 ± 10.31 μmol/L) than in healthy controls (21.80 ± 5.47 μmol/L) (P < 0.001) **Table (2).**

Table (2): Comparison of mean homocysteine serum level between BD patients and healthy controls:

Variable	Cases	Controls	P value
Homocysteine level (μmol/L) (Mean±SD)	37.87 ± 10.31	21.80 ± 5.47	< 0.001*

P-value > 0.05 (Non-significant)

Figure (1): frequency of different forms of ophthalmological affection in studied Behçet's disease patients.



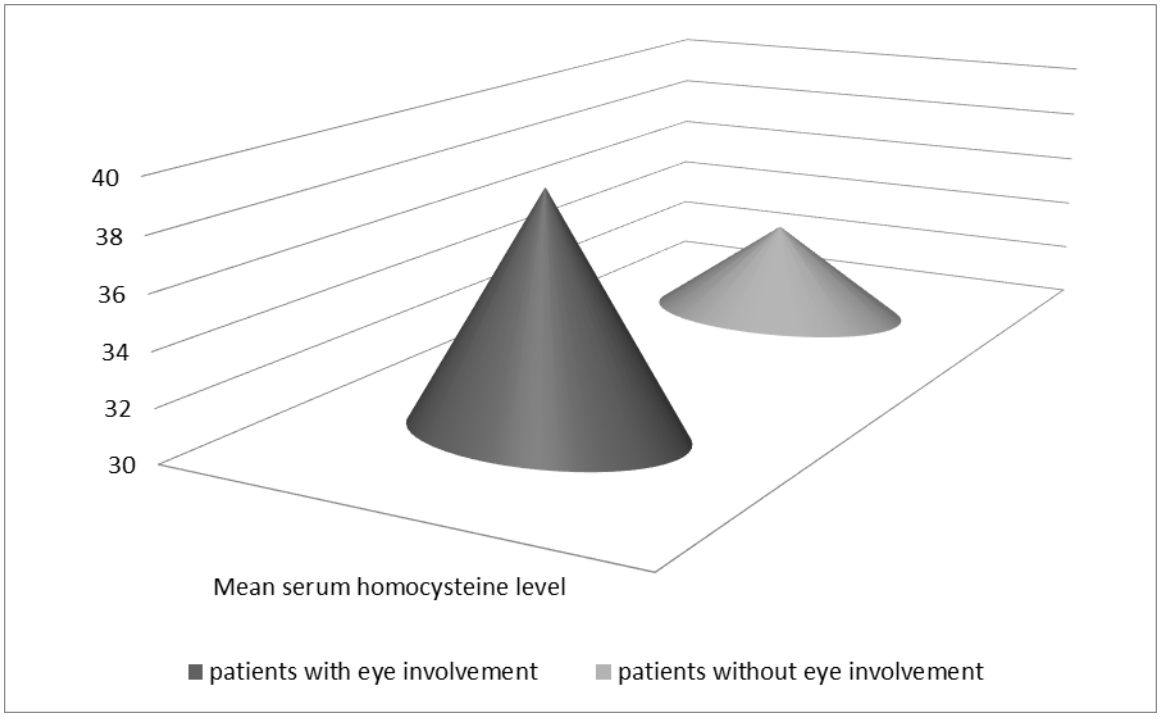
Patients with eye involvement had greater HCY levels than those who did not have eye involvement, yet, these differences did not attain a statistically significant level (P = 0.278) **Table (3).**

Table (3): Comparison of mean homocysteine serum level between patients with and patients without eye involvement

Variable	Patients with eye involvement	Patients without eye involvement	P value
Homocysteine level Mean ± SD (μmol/L)	38.91 ± 10.13	33.72 ± 10.9	0.278

P-value > 0.05 (Non-significant)

Figure (2): Homocysteine levels in the blood in patients with and without eye problems.



patients with eye involvement included 19 males (79.2%) and 5 females (20.8%). Males were significantly more common than females (P<0.001) with male to female ratio of 3.8:1. While all patients without eye involvement were males. Although mean serum HCY level was

relatively greater in females than in male patients with eye involvement, these differences were insignificant statistically (P>0.05) **Table (4).**

Table (4): Mean Homocysteine serum level in males and females with and without eye involvement

Variable	With eye involvement (n=24)			Without eye involvement (n=6)		
	Males	Females	P value	Males	**Females**	**P value**
Homocysteine level Mean± SD (µmol/L)	37.23±10.54	45.30±5.05	0.114	33.72±10.9	-	**-**

P-value > 0.05 (Non-significant)

In patients group with eye involvement, patients with history of vascular affection (about four patients (13.3%) had vascular involvement: three patients (75%) had deep vein thrombosis (DVT) and one patient (25%) had abdominal aortic aneurysm) were found to have significantly higher mean serum HCY level than those with vascular affection in patients group without eye involvement **(P= 0.046).** Otherwise, no significant difference was found when mean serum HCY levels in patients with other organs were compared between both groups **Table (5)**

Table (5): Comparison of mean serum homocysteine levels in different organs affection between patients with and patients without eye involvement

Organ involvement	Patients with eye involvement (n=24)	Patients without eye involvement (n=6)	P value
Oral ulcers			
Number	24	6	
Homocysteine level	38.91±10.13	33.72 ± 10.9	0.278

Mean± SD (µmol/L)			

Genital ulceration

Number	22	6	
Homocysteine level Mean± SD (µmol/L)	40.45±8.68	33.72±9.95	0.114

Skin lesions

Number	11	3	
Homocysteine level Mean± SD (µmol/L)	36.98±11.44	35.7±6.02	0.858

Joint involvement

Number	5	2	
Homocysteine level Mean± SD (µmol/L)	38.84±8.23	28.8±9.9	0.221

Positive pathergy test

Number	4	1	
Homocysteine level Mean± SD (µmol/L)	42±7.97	41.1	0.836

Vascular involvement

Number	3	1	
Homocysteine level Mean± SD (µmol/L)	31.17±4.71	18.9	0.046

Neurological involvement

Number	1	2	
Homocysteine level Mean± SD (μmol/L)	36.3	38.15±10.35	0.842

GIT involvement

Number	1	0	
Homocysteine level Mean± SD (μmol/L)	22.5	-	-

P-value > 0.05 (Non-significant)

4. Discussion:

Behçet syndrom is a disorder having a diverse set of clinical signs and symptoms and progression[14] In this study, among patients with eye involvement, males (79.2%) were significantly more common than females (20.8%) **(P<0.001).** The ratio of males to females was 3.8:1. This finding agrees with the results of ***Cansu et al*** study in ***2016*** which discovered that males were more likely to have ocular involvement (P=0.014) [15]. Another study with a similar result concluded that Men were more likely than women to have all types of ocular abnormalities (among other illness symptoms) [16].

In the current study, the level of HCY in the blood was reported to be significantly greater in BD patients (37.87 μmol/L ± 10.31) compared to healthy controls (21.80 μmol/L ± 5.47) (P < 0.001). This result is comparable to what was discovered previously, Aksu et al in 2001 compared eighty-four patients with BD to thirty-six age and sex-matched healthy controls where they came to the conclusion that patients' HCY serum levels were significantly more than controls (P<0.001) [17].

Er et al., 2002 study also had been conducted on 43 consecutive BD patients, and 25 healthy control volunteers of a similar age and sex. The overall mean HCY levels in the blood were considered to be significantly greater in people with BD compared to healthy people (P <0.001) [7].

Another study was done between January 1999 and May 2001, involved 90 consecutive cases already being followed, along with newly diagnosed patients. HCY levels in the plasma were observed to be higher in BD patients than in healthy controls (16.08 μmol/L ± 7.5 vs. 12.9

μmol/L ± 6.3 [18]. Differences in the HCY serum level between our study and other studies may be related to racial variations, disease activity, associated comorbidities, other factors that influence the level of HCY in serum such as vitamin B12 and folate deficiency and the technique used ELIZA or Chemiluminescence immunoassay [21].

Similarly, in ***(Houman et al., 2003)*** study which included 59 healthy controls and fifty-nine BD patients, the median HCY total levels and hyperHCY prevalence in the patients' group (13.3 μmol/L and 16.9% respectively) in comparison to the control group, were significantly higher (10.9 μmol/L and 5% respectively) [19].

Ates et al had investigated hyperHCY, being a well-known thrombosis risk factor, In BD, it's also a major risk for arterial and venous thrombosis. HCY serum levels were much more in individuals with BD than in healthy individuals according to a fluorescence polarisation immunoassay (P<0.01) [20**].**

The same was found by ***Sarican et al*** in ***2007*** where sixty-four patients with BD were enrolled and a negative control group of twenty-six healthy people was included in the study. Chemiluminescence immunoassay was used to assess the total serum HCY levels. Total BD patients had considerably higher mean serum HCY concentrations than healthy controls. (11.7 μmol/L ±4.6 versus 8.7 μmol/L ±2.8, P<0.01) [21].

In this work, although HCY levels were greater in patients with ocular involvement than in those without, there were no notable differences (P>0.05).

This is similar to what was found by ***Elbay et al*** [22] where they had found in Behcet uveitis group HCY levels in serum were 15.04±4.59 μmol/L while in the non Behçet uveitis group it was 15.4± 6.87 μmol/L (P>0.05)

Hamzaoui and her colleagues **in 2010** [23] also had reported that there was no link between serum HCY levels and disease manifestations such as retinal vasculitis(P=0.06) or uveitis (P=0.08) .

Aflaki et al in ***2008*** had conducted a study on 150 patients diagnosed to have BD. Similar to our results, they had found that there were no statistically significant variations in HCY serum levels between patients with and without eye involvement [24].

In contrast to our result, ***(Er et al., 2002)*** study had concluded that when compared to non-ocular BD participants, patients with ocular BD have considerably higher total HCY levels. (P<0.001). This can be explained by that in ***Er et al*** study none of the patients or controls had received any topical or systemic treatment for at least 2 weeks prior to blood collection**,** which may have led to increased activity of the disease, which in turn, as was found by the same study, was correlated with serum HCY level [7].

Yesilova et al also had concluded that, Patients with eye involvement had considerably greater serum total HCY concentrations than those without. The length of the disease ranged from 6 to 26 years (mean 75.56±71.72 months). This is longer than the disease duration of patients included in our study. This difference in disease duration may explain the conflicting results [11].

Regarding the relationship between HCY serum levels and the features of patients in various categories, in individuals with or without ocular involvement, there were no statistically significant relationships between mean serum HCY levels and age or disease duration P> 0.05). Also ***Allam et al*** **t**here was no link between serum HCY levels and duration of BD patients or age [25]. While ***Ates et al*** in ***2005***, had discovered that the serum mean HCY level was significantly correlated with age but not with the length of the condition in BD patients [26].

Our study showed that in patients with Behcet's disease, serum homocysteine mean levels are considerably higher than in healthy controls. However, there were no significant variations in homocysteine serum levels between patients who had ocular involvement and those who did not.

5. Recommendations and Conclusion:

In BD, the level of homocysteine in the serum is elevated. Homocysteine levels were shown to have no link to eye involvement. The study recommend measurement and monitoring of Hcy levels Because hyperhomocysteinemia is a treatable risk factor, it could be a useful index in the study, care, and improvement of BD patients' outcomes.

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