# Assessment of 25-hydroxyvitamin D level in patients with Behçet's Disease and its correlation with disease activity and severity

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#### Objective

The aim of this study was to assess the level of vitamin D in Behçet's disease (BD) patients and in healthy controls, and to correlate its level with clinical and laboratory data as well as disease activity and severity.

## Patients and methods

Forty patients with BD and 40 age-matched and sex-matched healthy controls participated in this study. Serum 25-hydroxyvitamin D [25(OH)D] was estimated using enzyme-linked immunosorbent assay in both patients and controls. Behçet's Disease Current Activity Form 2006 was used to assess disease activity. Disease severity was evaluated in BD patients.

#### Results

The mean 25(OH)D level in BD patients was lower than that in the control group, but with no statistical significance (P>0.05). The frequency of vitamin D deficiency was (27.5%) in BD patients whereas in controls, it was (7.5%), and the frequency of normal vitamin D level in BD was (2.5%) whereas in controls, it was (15%); the difference between the patients and controls was statistically significant (P=0.006). We found a significant negative correlation between the serum vitamin D level in BD patients and disease duration (r=-3.38; P=0.015). No significant correlation was found between the 25(OH)D level and disease activity of BD patients (P>0.05). According to the level of vitamin D, we classified our patients into three groups: normal (>30 ng/ml), insufficient (10–30 ng/ml), and deficient (<10 ng/ml) vitamin D level. A statistically significant difference was found between the three groups of BD patients with regard to the serum calcium level (P=0.03) and disease severity (P=0.028).

#### Conclusion

Vitamin D was lower in BD patients than in the healthy control group; hence, assessment of its level should be carried out in all patients with BD. Furthermore, vitamin D could be used as a new marker for disease severity in BD.

#### Keywords:

autoimmune diseases, Behçet's disease, vitamin D

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# Introduction

Behçet's disease (BD) is a systemic vasculitis of unknown cause, characterized by recurrent oral and genital ulcers and uveitis. Cutaneous, articular, neurological, intestinal, pulmonary, or vascular manifestations have also been reported [1].

Although the pathogenesis of BD remains unclear, many factors have been implicated. Heredity, immunologic factors, inflammatory mediators, viral antigens, and bacterial antigens, when superimposed into a favorable genetic background, can all play a role in the pathogenesis [2].

Vitamin D is a steroid hormone playing a major role in the maintenance of proper bone metabolism [3]. The active form of vitamin D,  $1,25(OH)_2D_3$  is required for its

physiological function, regulation of calcium and phosphorus uptake from the gut and kidney, thus providing the substrate for proper bone mineralization [4].

However, the discovery of the vitamin D receptor (VDR) in 1974 in nonskeletal tissue, particularly in immune cells, such as lymphocytes and monocytes indicated a possible role for vitamin D in maintaining normal function of the immune system [5].

Interestingly, vitamin D deficiency or reduced intake has been linked to an increased susceptibility to develop

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autoimmune diseases such as rheumatoid arthritis [6], systemic lupus erythematosus [7], multiple sclerosis [8], type 1 diabetes [9], vasculitis [10], and BD [11].

The effect of vitamin D on the immune system is an enhancement of the innate immunity coupled with multifaceted regulation of adaptive immunity [12]. Vitamin D has numerous effects on cells within the immune system. It inhibits B-cell proliferation and blocks B-cell differentiation and immunoglobulin secretion [13]. Vitamin D additionally suppresses T-cell proliferation [14] and results in a shift from a T-helper type 1 (Th1) to a Th2 phenotype [15]. Furthermore, it affects T-cell maturation with a skewing away from the inflammatory Th17 phenotype [16] and facilitates the induction of T regulatory (Treg) cells [17]. These effects result in the decreased production of inflammatory cytokines [interleukin-17 (IL-17), IL-21] with increased production of anti-inflammatory cytokines such as IL-10.

Vitamin D also has effects on monocytes and dendritic cells. It inhibits monocyte production of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, and tumor necrosis factor- $\alpha$  [18]. It additionally inhibits dendritic cell differentiation and maturation with preservation of an immature phenotype as evidenced by a decreased expression of major histocompatibility complex class II molecules, costimulatory molecules and IL-12 [19].

The data relating vitamin D to autoimmune and inflammatory diseases are equivocal with studies linking low vitamin D levels to dysregulation of Th1/Th2 and Th17/Treg ratios [20].

Th1, Th2 cells, and Treg cells have been shown to express the VDR and to be vitamin D targets [7]. In BD, vitamin D could be considered as an important mediator, the fluctuation of which is correlated to the inflammatory state of the disease [21].

During the BD-active phase, T cells and particularly CD4<sup>+</sup> lymphocytes are intensively stimulated and switched from naïve to memory CD4<sup>+</sup> T cells [22]. It has been reported that quiescent CD4<sup>+</sup> T cells express VDRs at low concentrations, which increase five-fold after their activation [23]. The explanation for the decreased vitamin D in the active stage was that CD4 cells consumed intrinsic vitamin D levels during their activation [20].

Do *et al.* [24] demonstrated a higher constitutive expression of Toll-like receptor 2 and Toll-like receptor 4 in the blood monocytes derived from BD

patients compared with healthy controls. Furthermore, it has become clear that the susceptible infection of *Streptococci* and *Mycoplasma*, and immunological cross-reaction with Heat Shock protein 60 (HSP60) act as a "Danger signal" in the initiation and progression of BD [25].

According to Hamzaoui *et al.* [11], 25-hydroxyvitamin D [25(OH)D] values were significantly lower than those of healthy controls. When vitamin D was measured according to clinical BD activity, lower serum vitamin D levels were found in active BD patients compared with inactive BD patients and healthy controls. Both Hamzaoui *et al.* [11] and Do *et al.* [24] reported that the serum vitamin D level was negatively correlated with the serum C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) in active BD patients. Their results suggested that disease activity is associated with lower vitamin D serum levels in active BD patients.

According to disease severity, Hamzaoui *et al.* [11] reported that the level of vitamin D was significantly lower in BD patients with pulmonary involvement or neurological manifestations compared with other patients.

Faezi *et al.* [26] did not reveal a significant correlation between disease activity or severity and vitamin D level. However, it is to be noted that BD cases with severe activity were excluded in accordance with the instructions of their study; therefore, BD was silent in most of the patients (80%) at the time of the study.

Given the importance of vitamin D for a functional immune system and the deficiency observed in autoimmune disease, as well as the correlation of deficiency with more active disease, the aim of this study was to assess the level of vitamin D in BD patients and in healthy controls, and to correlate its level with disease activity and severity.

# Patients and methods

Forty patients with BD (36 male and four female) diagnosed according to the International Study Group Criteria for the classification of BD [27] and 40, agematched and sex-matched healthy volunteers serving as the control group were included in this study. They were conveniently recruited from the inpatient department and the outpatient clinic of the Department of Rheumatology and Rehabilitation, Kasr El-Aini Hospitals, Cairo University, over a period of 18 months from January 2013 to June 2014. Their ages ranged from 20 to 70 years, with a mean of 39.08 years.

Exclusion criteria were the use of vitamin D supplements in the past 6 months, pregnancy, chronic renal failure, liver disease, and thyroid and parathyroid disorders.

This study was approved by the local ethics committee of Cairo University scientific review board, and informed consent was obtained from all patients according to the Declaration of Helsinki; General Assembly, October 2008.

All patients were subjected to the following assessments:

- (1) Full history taking and thorough clinical examination.
- (2) Behçet's Disease Current Activity Form (BDCAF) 2006 [28] to assess disease activity. Scoring was based on the history of new clinical features present over the 4 weeks before assessment. Only clinical features that the clinician felt were due to BD should be scored. BDCAF scores include the following:
  - (a) Headache.
  - (b) Mouth ulceration.
  - (c) Genital ulceration.
  - (d) Erythema.
  - (e) Skin pustules.
  - (f) Joints arthralgia.
  - (g) Joints arthritis.
  - (h) Nausea/vomiting/abdominal pain.
  - (i) Diarrhea+altered/frank blood per rectum.
  - (j) Eye involvement.
  - (k) Nervous system involvement (include intracranial vascular disease).
  - (l) Major vessel involvement (exclude intracranial vascular disease).

Patients index score ranges from 0 to 12. A score of 1 is given to each item present over the last 4 weeks. You should have a score out of 12, which is the patient's BD Activity Index Score.

(3) Assessment of disease severity according to Krause *et al.* [29]:

The severity score was calculated as the sum of one point for each of mild symptoms, two points for each of moderate symptoms, and three points for each of severe disease manifestations.

- (a) Mild symptoms:
  - (i) Oral aphthosis.

- (ii) Genital ulcers.
- (iii) Typical skin lesions (erythema nodosum, papulopustular lesions, folliculitis, leukocytoclastic vasculitis).
- (iv) Arthralgia.
- (v) Recurrent headaches.
- (vi) Epididymitis.
- (vii) Mild gastrointestinal symptoms (chronic diarrhea, chronic recurrent colicky abdominal pain).
- (viii) Pleuritic pains.
- (ix) Superficial vein thrombosis.
- (b) Moderate symptoms:
  - (i) Arthritis.
  - (ii) Deep vein thrombosis of the legs.
  - (iii) Anterior uveitis.
  - (iv) Gastrointestinal bleeding.
- (c) Severe symptoms:
  - (i) Posterior/pan uveitis, retinal vasculitis.
  - (ii) Arterial thrombosis or aneurysm.
  - (iii) Major vein (vena cava, hepatic) thrombosis.
  - (iv) Neuro-Behçet.
  - (v) Bowel perforation.
- (4) Laboratory investigations including complete blood count, ESR, liver function tests, serum urea and creatinine, total serum calcium, phosphorus and alkaline phosphatase, and complete urine analysis.
- (5) Total serum 25(OH)D, as a measure of vitamin D status, was assessed by enzyme-linked immunosorbent assay (ELISA), using a commercial kit from DRG International Inc. (Germany), which has a DIN ISO 9001 (2000) certification. The DRG 25(OH)D total ELISA kit is a solid-phase ELISA, based on the principle of competitive binding.

In the first step, samples were pretreated in separate vials with denaturation buffer to extract the analyte, as most circulating 25(OH)D is bound to VDBP in vivo. After neutralization, biotinylated 25 (OH)D (enzyme conjugate) and peroxidase-labeled streptavidin-(enzyme complex) were added. After careful mixing, the solution was transferred to the wells of the microtiter plate. Endogenous 25(OH)D of a sample competes with a 25(OH)D-biotin conjugate for binding to the VDBG that was immobilized on the plate. Binding of 25(OH)D-biotin was detected by peroxidase-labeled streptavidin. Incubation was followed by a washing step to remove unbound components. The color reaction was started by the addition of enzyme substrate and stopped after a defined time. The color intensity was inversely proportional to the concentration of 25(OH)D in the sample.

Clinical manifestations	N (%)
Oral ulcers	40 (100)
Genital ulcers	40 (100)
Eye manifestations	28 (70)
Skin manifestations	12 (30)
Arthralgia	15 (37.5)
Arthritis	7 (17.5)
Neurological manifestations	3 (7.5)
GIT manifestations	10 (25)
Vascular manifestations	11 (27.5)
GIT. gastrointestinal tract.	

Table 3 Laboratory data of Behçet's disease patients

Laboratory data	Behçet's patients		
	Range	Mean±SD	
ESR (mm/1st hour)	2–125	29.8±28	
Hemoglobin (g/dl)	8–17.6	13.4±2.06	
Total leukocyte count (thousand/mm <sup>3</sup> )	4–18	9.5±3.49	
Platelet count (thousand/mm3)	172–385	262.4±55.04	
Serum albumin (mg/dl)	3.1–4.9	4.23±0.44	
Serum Ca (mg/dl)	7.5–10.2	9.03±0.61	
PO <sub>4</sub> (mg/dl)	1.3–5.1	3.3±0.8	
Serum creatinine (mg/dl)	0.5–1.2	0.7±0.16	
ALT (IU/I)	7–316	43±55	
AST (IU/I)	6–156	27±23	
ALP (IU/I)	43–211	85±34	

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; Ca, calcium; ESR, erythrocyte sedimentation rate.

BD patients were divided according to the level of 25 (OH)D into three groups: normal (>30 ng/ml), insufficient (10–30 ng/ml), and deficient (<10 ng/ml) vitamin D level.

## Statistical analysis

All statistical calculations were performed using computer program statistical package for the social science (SPSS Inc., Chicago, Illinois, USA) release 15 for Microsoft Windows (2006). Data were statistically described in terms of mean±SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was performed using the Student *t*-test for independent samples in comparing two groups when normally distributed and Mann-Whitney U-test for independent samples when not normally distributed. Comparison of numerical variables between more than two groups was performed using one-way analysis of variance test with post-hoc multiple two-group comparisons in normal data and Kruskal-Wallis test in non-normal data. For comparing categorical data, the  $\chi^2$ -test was performed. Exact test was used instead when the expected frequency was less than 5.

Table 2 Behçet's Disease Current Activity Form of Behçet's disease patients

	N (%)
Headache	27 (67.5)
Active oral ulcers	22 (55)
Active genital ulcers	11 (27.5)
Arthralgia	7 (17.5)
Active arthritis	5 (12.5)
Active skin manifestations	8 (20)
Active GIT	11 (28)
Eye activity	26 (65)
CNS activity	1 (3)
Major vessel activity	0 (0)
BDCAF [mean (range)]	3±1.72 (0–6)

BDCAF, Behçet's Disease Current Activity Form; CNS, central nervous system; GIT, gastrointestinal tract.

Drugs	N (%)
Current oral corticosteroids	36 (90)
Endoxan	14 (35)
Imuran	19 (47.5)
Colchicine	20 (50)

Correlation between various variables was determined using the Pearson moment correlation equation for linear relation in normally distributed variables and Spearman's rank correlate on equation for non-normal variables and/or nonlinear monotonic relation. *P* values less than 0.05 were considered statistically significant.

## **Results**

Forty adult BD patients, 36 (90%) male and four (10%) female as well as 40 age-matched and sexmatched, healthy individuals serving as the control group were recruited for this study. The age of BD patients ranged from 21 to 70 years with a mean of  $35\pm10$  years. Age of onset ranged from 11 to 40 years with a mean of  $26.23\pm7.241$ years. Disease duration ranged from 1 to 34 years with a mean of  $8.925\pm7.55$ years. Sixteen (40%) of the BD patients were smokers. None of the patients was taking alcohol.

The clinical data of the BD patients are shown in Table 1.

Regarding BDCAF of BD patients, the total score of headache, oral ulcers, genital ulcers, arthralgia, arthritis, skin manifestations, gastrointestinal tract manifestations, eye activity, central nervous system activity, and major vessel activity, in the last month, ranged from 0 to 6 with a mean of 3±1.72 (Table 2).

Severity of BD was graded as mild, moderate, and severe according to Krause *et al.* [29]:

Table 6 State of 25 budgeyunitemin D in Debeat's disease nationts and as

Table 5 Serum	level of 25-h	ydroxyvitamin	D in	Behçet's	disease	patients	and	controls
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		25(OH)D (ng/ml)		
	Minimum	Maximum	Mean±SD	
BD patients (n=40)	1.2	51.6	16.78±9.15	0.062
Controls (n=40)	7.3	95.5	23.34±19.81	

25(OH)D, 25-hydroxyvitamin D; BD, Behçet's disease.

Table 6 State of 25-flydroxyvitallin D in Ber	içer s'disease patients and controis		
	BD patients [N (%)]	Controls [N (%)]	P-value
Vitamin D deficiency (<10 ng/ml)	11 (27.5)	3 (7.5)	0.190
Vitamin D insufficiency (10-30 ng/ml)	28 (70)	31 (77.5)	
Normal vitamin D level (>30 ng/ml)	1 (2.5)	6 (15)	

BD, Behçet's disease.

Table 7 Comparison between the number of Behçet's disease patients and controls with regard to normal and deficient 25hydroxyvitamin D level

	BD patients [N (%)]	Controls [N (%)]	P-value
Deficient vitamin D	11 (27.5)	3 (7.5)	0.006
Normal vitamin D	1 (2.5)	6 (15)	

BD, Behçet's disease.

Mild disease severity was found in four (10%) patients.

Moderate disease severity was found in seven (17.5%) patients.

Severe disease was found in 29 (72.5%) patients.

The laboratory data of the BD patients are shown in Table 3.

The medical treatment of the BD patients are shown in Table 4.

The mean 25(OH)D level in the BD patients was lower than in the control group, but with no statistical significance (16.78 $\pm$ 9.15 vs. 23.34 $\pm$ 19.81 ng/ml; *P*=0.062, respectively) (Table 5).

Eleven (27.5%) patients and three (7.5%) of the controls had deficient vitamin D levels, 28 (70%) patients and 31 (77.5%) of the controls had insufficient vitamin D levels, whereas one (2.5%) patient and six (15%) of the controls had normal serum vitamin D level. Insufficiency of 25 (OH)D was less common in the patients than in the control group, whereas deficiency was more common in patients than in the control group. On comparing the number of BD patients and the control group with regard to normal, insufficient, and deficient levels of 25(OH)D, we did not find a statistically significant difference (P=0.190) (Table 6).

On comparing the number of BD patients and the control group with regard to normal and deficient levels

of 25(OH)D, we found a statistically significant difference (P=0.006) (Table 7).

There was no statistically significant difference between male and female patients with regard to the level of 25(OH)D (*P*=0.787).

Correlations between 25(OH)D and the demographic data of BD patients showed a significant negative correlation with disease duration (*r*=-3.383; *P*=0.015), but not with age, age of onset, or smoking (*P*=0.443, 0.083, 0.646, respectively).

There was no statistically significant correlation between 25(OH)D level and the clinical features as well as various laboratory data of BD patients.

Neither disease activity (P=0.448) nor disease severity (P=0.656) of BD patients showed a significant correlation with the 25(OH)D level.

The 25(OH)D level showed no significant correlation with any specific medication received by the patients.

Accordingly, we divided the BD patients into three groups: normal, insufficient, and deficient vitamin D level.

We found no statistically significant difference between the three groups with regard to the age, sex, or disease duration of BD patients (P=0.704, 0.934, 0.162, respectively).

P-value

0.028\*

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Disease severity	Deficient (<10 ng/ml)	Insufficient (10-30 ng/ml)	Normal (>30 ng/ml)	Total				
Mild	0	3	1	4				
Moderate	3	4	0	7				
Severe	7	21	0	28				

Table 8	Comparison	between 1	the three	groups	with	regard to	the	disease	severity
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\*Significant at P<0.05.

Severe

Table 9 Comparison between the three groups with regard to the laboratory data

Laboratory data	Deficient (<10 ng/ml)	Insufficient (10-30 ng/ml)	Normal (>30 ng/ml)	Total	P-value
ESR	33.27±39.28	28.39±23.57		29.78±28.02	0.635
Hb	12.58±2.94	13.7±1.59		13.36±2.05	0.255
Serum creatinine	0.71±0.18	0.78±0.16		0.76±0.16	0.246
ALT	21.09±8.96	52.29±63.5		43.00±54.96	0.17
AST	19.73±8.33	29.68±27.18		26.65±23.48	0.244
Ca	8.69±0.70	9.16±0.54		9.03±0.61	0.03*
Ph	3.14±1.15	3.29±0.54		3.28±0.77	0.686
ALP	84.82±17.50	85.68±38.72		85.03±33.52	0.944
Vitamin D	6.83±2.45	19.44±5.12		16.78±9.15	0.00

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; Ca, calcium; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; Ph, phosphorus. \*Significant at P<0.05.

No statistically significant difference was found between the three groups with regard to the clinical features of BD patients.

On comparing the three groups with regard to disease activity, no statistically significant difference was found (P=0.770). However, there was a significant difference between them with regard to the disease severity (P=0.028) (Table 8).

On comparing the three groups with regard to the laboratory findings, we found a statistically significant difference between them with regard to the serum calcium level (P=0.03), but not with respect to other laboratory data (Table 9).

No statistically significant difference was found between the three groups with regard to the current dose of steroids (P=0.939), cumulative dose of steroids (*P*=0.69), or colchicine (*P*=0.303).

## Discussion

Vitamin D is one of the fat-soluble vitamins; its main source in humans are animal products and sun exposure, and is transformed into its active metabolite in the liver and kidney. The main function of vitamin D in the body is calcium homeostasis [3].

Vitamin D has multiple immunosuppressant properties. Its importance could be explained by previous reports stating that it decreases the incidence and/or progression of autoimmune diseases significantly [30,31].

The prevalence of vitamin D deficiency in various autoimmune diseases and the association between its deficiency and disease outcome is one of the most important evolving topics [32]. Our study aimed to asses serum levels of vitamin D in patients with BD and to evaluate its relationship with different disease parameters as well as disease activity and severity.

The results of our study demonstrated that the mean serum level of vitamin D was lower in BD patients compared with its level in the control group, which surprisingly lies in the insufficiency range despite the fact that our population resides in a North East African country with plenty of sunny days, and our control samples were taken in June (summer season). When comparing serum vitamin D levels of BD patients with healthy controls, the difference was statistically insignificant (P=0.062). This could be a result of the considerable prevalence of vitamin D deficiency and insufficiency in our control groups reflecting the poor health education in the general population, as well as other parameters such as lack of sun exposure or inadequate nutritional intake.

Alpsoy et al. [33] detected a lower level of vitamin D in BD patients in comparison with the control group. However, this difference was not significant, which agreed with the findings in our study. The study by Faezi et al. [26] showed a high prevalence of vitamin D deficiency in BD patients. However, in that study, on comparing the level of vitamin D in BD patients and controls, they found that BD patients had a significantly higher level of serum vitamin D, which was explained by the insightful education these patients obtain in Behçet's clinics.

In contrast, when we divided the patients and controls into those with normal and deficient vitamin D levels, one (2.5%) patient and six (15%) of the controls had normal vitamin D level, whereas 11 (27.5%) patients and three (7.5%) of the controls had deficient vitamin D level; the difference between them was statistically significant (P=0.006). This was in agreement with Ganeb *et al.* [34], who studied serum levels of vitamin D in 42 patients with BD and a control group of 41 age-matched and sex-matched individuals, and found that 17 (40.5%) patients and 28 (68.3%) of the controls had normal vitamin D serum levels, whereas 25 (59.5%) patients and 13 (31.7%) of the controls had low vitamin D levels, and the difference was statistically significant (P=0.011).

Concerning the demographic data of our BD patients, we found a significant negative correlation between the vitamin D serum level and disease duration (r=-3.383; P=0.015). This could be explained by the fact that persistent, uncorrected, deficient serum vitamin D levels in our BD patients could contribute to prolongation of disease duration owing to the presumable effect of vitamin D deficiency in maintaining the inflammatory state of the disease.

Also, Jassim *et al.* [35] found that serum vitamin D mean levels in their study on 42 BD patients were significantly inversely correlated with BD duration. However, other studies [26,34,36] showed no significant correlation between vitamin D level and disease duration in their BD patients. According to Jassim *et al.*, [35] this is probably because of the difference in the number of patients included in each study.

In our study, there was a nonsignificant correlation between vitamin D levels and the age of BD patients (P=0.443). This agreed with the study conducted by Faezi *et al.* [26] and Karatay *et al.* [36]. In contrast, a significant negative correlation was found between vitamin D serum level and patients' age in other studies [11,37,38]. Lerner *et al.* [39] reported that age was the major factor affecting serum vitamin D levels. Yu and Cantorna [40] stated that in aged BD patients, the calcium status of the host may influence the effect of vitamin D on immunity. This could explain the inverse correlation observed in aged BD patients.

In our study, we found no significant correlation between vitamin D levels in BD patients and smoking (P=0.646). In contrast, Karatay *et al.* [36] considered smoking to be one of the main predictors of 25(OH)D level in their study.

The results of our study exhibited a nonsignificant correlation of vitamin D serum levels with BD

activity (P=0.448). This was in agreement with Faezi *et al.* [26] who found no significant relationship between disease activity and vitamin D level. In a study by Khabbazi *et al.* [41], aiming to examine the status of vitamin D in patients with active and recently diagnosed BD, and the relationship between vitamin D level and BD activity, they demonstrated that the mean 25(OH)D level in the BD group was lower than that in the control group. Insufficiency and deficiency of 25(OH)D was more common in the BD group than in the control group. No correlation was observed between the Iranian Behçet's Disease Dynamic Activity Measure or the BDCAF and 25(OH)D level. They suggested that deficiency of 25(OH)D may be a trigger factor for BD.

Do *et al.* [24] showed decreased serum vitamin D levels in active BD patients compared with controls and suggested that vitamin D deficiency is a possible risk factor for BD activity and the disturbance in the inflammatory condition. However, the difference between the active BD group and the inactive BD group with regard to vitamin D levels was not significant. In contrast, Hamzaoui *et al.* [11] reported a lower level of vitamin D in BD-active patients compared with patients with inactive disease and healthy controls (P=0.0246, 0.0001, respectively).

Ganeb *et al.* [34] aimed in their study to investigate serum levels of vitamin D in BD and to evaluate its relationship with disease activity as well as different disease measures. They reported that serum levels of vitamin D was significantly lower in BD patients compared with controls. They found a significant negative correlation of serum vitamin D levels with BDCAF and concluded that low vitamin D may predispose BD patients to active disease, especially in older individuals.

When we studied the correlation between vitamin D levels in BD patients and disease severity, it was insignificant (P=0.656), a result that agreed with Faezi *et al.* [26] who studied 112 BD patients (46 male and 66 female) and 112 healthy individuals (22 male and 90 female), and also found no significant correlation.

However, in our study, on comparing BD patients with normal, insufficient, and deficient serum vitamin D level with regard to the severity of the disease, we found a statistically significant difference between the three groups (P=0.028). Also, Hamzaoui *et al.* [11] found that the vitamin D level was significantly lower in BD patients with pulmonary involvement or neurological manifestations. Ganeb *et al.* [34] observed a statistically significant decrease of vitamin D serum levels in BD patients with vascular lesions only.

In our study, there was no significant correlation between serum vitamin D levels in BD patients and ESR or CRP. This may be due to the fact that not all Behçet's patients had active disease at the time of recruitment. These findings were in agreement with those of Karatay *et al.* [36] who also reported similar results. In contrast, the serum vitamin D level was inversely correlated with ESR and the CRP level in BD in other studies [11,24,34].

According to the study conducted by Karatay *et al.* [36], a nonsignificant relation was found between vitamin D level and the level of serum calcium and phosphorus, which agreed with the findings in our study. In contrast, in our study, when patients were divided into three groups according to the vitamin D level, there was a significant difference between the three groups of BD patients with regard to the serum calcium level (P=0.03).

Our study revealed no significant correlation between serum vitamin D levels in BD patients and corticosteroid treatment (P=0.429), which was in agreement with Karatay *et al.* [36]. Also, Faezi *et al.* [26] reported that 37% of BD patients had a history of corticosteroid administration; the analysis revealed no significant difference in vitamin D levels between patients who had received corticosteroids and patients who had not. They explained this finding by the fact that their patients discontinued corticosteroids during a 6-month period before his study.

In the present study, no significant correlation was detected between vitamin D level in BD patients and colchicine treatment (P=0.440). Ganeb *et al.* [34] reported that colchicine therapy was one of the independent effectors on vitamin D serum level in multivariate regression analysis. In their study, Karatay *et al.* [36] concluded that the most effective predictor of vitamin D level in BD patients was colchicine.

In conclusion, the vitamin D level was not significantly decreased in patients with BD as compared with controls, although the frequency of vitamin D deficiency in BD patients in comparison with controls was significant. Future studies on a larger number of patients are needed to detect whether vitamin D could be used as a marker for disease severity in BD. Financial support and sponsorship  $Nil. \label{eq:nonlinear}$ 

## **Conflicts of interest**

There are no conflicts of interest.

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