Aim of the work: This study aimed to investigate serum levels of vitamin D in patients with Behçet’s disease (BD) and to evaluate their relationship to disease activity as well as different disease measures.

Patients and methods: Forty-two patients with BD were enrolled into this study. These patients were subjected to detailed history taking, thorough clinical examination including assessment of disease activity according to Behçet’s Disease Current Activity Form (BDCAF) score and performed laboratory investigations including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum calcium, serum phosphorus and serum alkaline phosphatase. Serum 25-hydroxyvitamin D (vitamin D) levels were determined using Enzyme-Linked-Immunosorbent Assay (ELISA). A control group of 41 age and sex matched healthy controls was also included.

Results: The mean level of 25-hydroxyvitamin D (30.65 ± 12.87 ng/ml) was significantly decreased in BD patients compared to the controls (37.98 ± 15.76 ng/ml) ($p = 0.02$). Significant negative correlations of serum vitamin D levels with patients’ ages ($p = 0.03$), ESR ($p < 0.001$), CRP ($p < 0.001$) and BDCAF ($p = 0.003$) were found; whereas, there was no significant correlation with disease duration ($p = 0.6$). In multivariate regression analysis age ($p = 0.02$), colchicine
1. Introduction

Behcet’s disease (BD) is a systemic relapsing chronic inflammatory disease of unknown etiology that presents with recurrent oral ulcers, genital ulcers, skin lesions and uveitis [1–3]. A wide range of clinical features are observed, including involvement of the ophthalmic, musculoskeletal, vascular, central nervous and gastrointestinal systems [4].

The pathogenesis of BD includes immune-mediated mechanisms and inflammatory mediators, hypersensitivity of T lymphocytes produced in response to different types of antigens has a critical role in the pathogenic mechanisms [5–7].

Vitamin D has a major function in maintaining normal calcium and phosphorus blood levels. The best measure of vitamin D availability is serum levels of 25-hydroxyvitamin D [8] which is the major circulating form, biologically inactive with a half-life of about two weeks and reflects the amount of vitamin D entering the circulation that proportionates to the amount of vitamin D ingested and produced in the skin [9–11]. It has major biologic activities including cellular proliferation and differentiation, immune system modulation and muscle strengthening [12–15].

Vitamin D is regarded as an environmental factor essential in the etiology of T-cell mediated autoimmune diseases [16]. Poor vitamin D status is important in the initiation and propagation of a range of autoimmune diseases [17] like multiple sclerosis, type 1 diabetes, systemic lupus erythematosus, and rheumatoid arthritis and is associated with an increased risk of several diseases such as hypertension, infectious diseases, diabetes, cardiovascular disease, musculoskeletal disorders, asthma, as well as several psychiatric conditions such as schizophrenia, depression, and dementia and solid tumors [18–20]. Few studies have evaluated serum levels of vitamin D in BD.

Some patients with autoimmune diseases have anti-vitamin D antibodies while, several autoimmune pathways are suppressed by vitamin D including the Th1, B cells, Th-17, dendritic cell, and co-stimulatory molecule systems [21]. Vitamin D metabolites have direct targets to the adaptive immune system cells [22].

The aim of this study was to investigate serum levels of vitamin D in patients with BD and to evaluate their relationship to disease activity as well as different disease measures.

2. Patients and methods

2.1. Subjects

Forty-two BD patients diagnosed according to the International Study Group Criteria for BD (ISGC 1990) [23] and forty-one age and sex matched healthy volunteers as control group were included in this study. They were recruited from the inpatient and outpatient clinics of Rheumatology, Rehabilitation & Physical Medicine, General Medicine, Ophthalmology and Dermatology & Andrology of Benha University hospitals in the period between January 2010 and July 2010.

Patients were excluded from the study if they had other illnesses that might affect the results of the study such as chronic liver, kidney or endocrine disorders. Subjects taking medications known to influence calcium, vitamin D, or phosphorus metabolism as anticoagulants; anticonvulsants; fluoride; anti-hypertensive drugs such as B-blockers, diuretics, and calcium channel blockers were also excluded.

Informed written consents were obtained from all participants and the study was approved by the local Ethics Committee.

2.2. Clinical assessments

All BD patients were subjected to the following:

- Detailed history taking
- Thorough clinical examination with stress on dermatological, locomotor, vascular and ophthalmologic examination.
- Skin pathergy test was done [24].
- Weights and heights of all subjects were recorded, and body mass index (BMI) was calculated as weight (kg)/height (m²).
- Assessment of disease activity according to Behcet’s Disease Current Activity Form (BDCAF) score [25].

2.3. Biochemical assessments

- Laboratory investigations: venous blood samples were obtained from all patients and controls. The following assessments were done:

  - Complete blood picture by coulter counter.
  - Erythrocyte sedimentation rate (ESR) by the Westergren method recorded in mm/1st h.
  - C-reactive protein (CRP) by standard nephelometry with an established normal range of 0–0.8 mg/l.
  - Total serum calcium (\(n = 8.1–10.4\) mg/dl), total serum phosphorus (\(n = 3.5–5\) mg/dl) and total serum alkaline phosphatase (\(n = 65–110\) IU/L) were assessed spectrophotometrically on Hitachi 91.
  - Total serum 25-hydroxyvitamin D (vitamin D) was assessed by Enzyme -Linked- Immunosorbent Assay (ELISA). The kit was derived from Immundiagnostik AG company had a DIN IBO 9001 (2000) certification (Australian).
Serum vitamin D levels >30 ng/ml were considered sufficient, while levels of 30 ng/ml or less were considered low.

Statistical analysis: Statistical analysis was performed using Statistical Package for Social Sciences software (SPSS), version 13.0. Quantitative variables were presented as the mean ± SD while qualitative data were expressed as number and percent. Student’s (t) test, Z, X², Pearson’s correlation coefficient (r) and Mann Whitney U (MWU) tests were used when appropriate. Multivariate regression analysis was done to evaluate the effects of different variables on vitamin D levels. p Value less than 0.05 was considered significant.

3. Results

3.1. Demographic and BD related variables

- Patients’ characteristics are represented in (Table 1): The mean disease duration was 9.77 ± 8.5 years. One patient (2.4%) was on steroid use of 20 mg/d for the last two months and 36 (85.7%) patients were on current colchicine therapy (ranging between 1–1.5 mg/d), two of them were taking low-dose steroids along with colchicine. Three patients (7.1%) were on current calcium and vitamin D supplementation. None of the patients was on bisphosphonate therapy.

- The mean BDCAF was 4.5 ± 2.9.

- Patients with BD presented with skin lesions as erythema nodosum, sterile pustules or papules in 16 patients (38.1%). Arthritis was present in 14 patients (33.3%). Ocular disorders [uveitis in 11 patients (26.2%), unilateral optic neuritis in one patient (2.4%) and retinal vasculitis in one patient (2.4%)] affected 13 patients (31%). Vascular lesions [venous thrombosis in three patients (7.1%) and arterial thrombosis in one patient (2.4%)] occurred in four patients (9.5%) (Table 2).

- As regards inflammatory markers, both ESR and CRP were significantly higher in patients (26.43 ± 14.43 mm/1st h) and (10.56 ± 8.87 mg/l) than controls (13.35 ± 5.74 mm/1st h) and (4.56 ± 3.57 mg/l), respectively (Table 3).

3.2. Vitamin D status in BD patients and controls

- There was a statistically significant decrease in serum vitamin D levels (p = 0.02) in patients (30.65 ± 12.87 ng/ml) compared to controls (37.98 ± 15.76 ng/ml).

- There was a statistically significant decrease (z = 2.16, p = 0.03) of the median values and IQ ranges of serum vitamin D levels between BD patients (29 and 22.75–39) and controls (36 and 25.5–48.5), respectively.

- On comparison of serum vitamin D levels according to gender in BD patients and control group, there were no significant differences of vitamin D levels according to gender in BD patients [Males: 30.89 ± 11.87; Females: 29.685 ± 12.87 (t = 0.31, p = 0.76)], or in healthy controls [Males: 38.76 ± 11.67; Females: 36.48 ± 11.65 (t = 0.62, p = 0.54)].

- A statistically significant decrease of vitamin D serum levels was observed in BD patients with vascular lesions (t = 3.72, p = 0.001), while there was an insignificant decrease of serum vitamin D levels in patients with oral ulcers (t = 1.5, p = 0.14), skin lesions (t = 0.5, p = 0.62), arthritis (t = 0.55, p = 0.58), ocular lesions (t = 0.28, p = 0.79) and positive skin Pathergy test (t = 0.82, p = 0.42) (Table 4).

- In the group with sufficient serum vitamin D levels, BD patients had significantly lower vitamin D levels compared to controls (40.4 ± 6.9 ng/ml and 47.8 ± 12.5 ng/ml, respectively, p = 0.03). In the group with low vitamin D,
patients had also significantly lower serum level of vitamin D compared to the control group (19.6 ± 6.7 ng/ml and 24.2 ± 4.6 ng/ml, respectively, \( p = 0.034 \)). Seventeen patients (40.5%) and 28 of controls (68.3%) had sufficient vitamin D serum levels, while twenty-five patients (59.5) and 13 of controls (31.7%) had low vitamin D levels and the difference was statistically significant (\( \chi^2 = 6.5, p = 0.011 \)) (Figure 1).

- Comparison of some clinical and laboratory variables in BD patients according to vitamin D levels revealed statistically significant differences as regards age (\( t = 2.15, p = 0.037 \)) and BDCAF (\( t = 2.77, p = 0.008 \)) (Table 5).

- On correlating vitamin D serum levels with some variables among controls, insignificant correlations were found with age (\( r = -0.18, p = 0.27 \)), ESR (\( r = -0.15, p = 0.36 \)) and CRP (\( r = -0.17, p = 0.28 \)).

3.3. Effectors of vitamin D levels in BD

- Significant inverse correlations of serum vitamin D levels with patients’ ages (\( r = -0.54, p = 0.03 \)), ESR (\( r = -0.59, p < 0.001 \)), CRP (\( r = -0.53, p < 0.001 \)) (Table 6) and BDCAF (\( r = -0.45, p = 0.003 \), Figure 2) were found. Whereas, there was insignificant correlation of serum vitamin D levels with disease duration (\( r = -0.1, p = 0.6 \)).

- Multivariate regression analysis was performed to determine the main effectors of vitamin D levels. The variables included age, disease duration, BDCAF, colchicine therapy, ESR and CRP. Age (\( p = 0.02 \), colchicine therapy (0.008), ESR (0.02) and CRP (0.03) were found to be the independent effectors of vitamin D levels (Table 7).

4. Discussion

The etiopathogenesis of BD is still unclear, many factors have been considered including: viral infection, autoimmune disease, streptococcal-related antigens and genetic factors, [26,27].

Vitamin D has multiple immunosuppressant properties [20/9]. Its importance could be explained by previous reports stating that it significantly decreases the incidence and/or progression of autoimmune diseases [28,29].

This study aimed to investigate serum levels of vitamin D in patients with BD and to evaluate their relationship to disease activity as well as different disease measures.
The results of our study demonstrated that serum levels of vitamin D were significantly decreased ($p = 0.02$) in BD patients compared to healthy controls. Seventeen patients (40.5%) and 28 of controls (68.3%) had sufficient vitamin D serum levels, while 25 patients (59.5%) and 13 of controls (31.7%) had low vitamin D levels and the difference was statistically significant ($p = 0.011$). Decreased serum levels of vitamin D have been reported in BD [30] and several other autoimmune diseases including rheumatoid arthritis [16,31], systemic lupus erythematosus [17,32], multiple sclerosis [33] and undifferentiated connective tissue disease [34]. Those studies suggested that down-regulation of vitamin D is almost involved in the pathogenesis of these diseases.

Colin et al. [31] stated that vitamin D and vitamin D analogs’ supplementation contribute to the bone-preserving effects and could have a role when combined with corticosteroids in the prevention of focal bone erosions in RA. They also reported that these findings may be effective for other chronic inflammatory autoimmune diseases treated with corticosteroids.

Several factors decrease vitamin D levels. Reduced skin synthesis and absorption of vitamin D occur with the aging process [32], obesity results in vitamin D sequestration in body fat [35], smoking impairs 1-alpha-hydroxylation [36] and enhances hepatic degradation of estrogen which alters metabolism of vitamin D [37], corticosteroids accelerate vitamin D catabolism [38] and estrogen deficiency increases parathormone hormone leading to decreased vitamin D [39].

This study revealed a statistically significant decrease of vitamin D serum levels in BD patients with vascular lesions compared to those patients without vascular lesions ($p = 0.001$). This confirmed previous studies that found vitamin D deficiency to be associated with cardiovascular events such as myocardial infarction or stroke [40,41].

We found a significant negative association between vitamin D serum levels and patients’ ages ($p = 0.03$). Several previous studies found a negative correlation between vitamin D serum levels and patients’ age [42–45]. Lerner et al. [21] reported that age is the major factor affecting serum vitamin D levels. Yu et al. [46] stated that in aged BD patients, calcium status of the host may influence the effect of vitamin D on immunity.

In the present study, there were significant negative correlations of vitamin D serum levels with BD patients’ ESR ($p < 0.001$) and CRP ($p < 0.001$). These findings were in agreement with those of Do et al. [30] who found decreased serum vitamin D levels were inversely correlated with ESR and CRP levels. On the other hand Karaty et al. [47] demonstrated that

<table>
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<tr>
<th>Table 5</th>
<th>Comparison of some variables in BD patients according to vitamin D serum levels.</th>
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<tbody>
<tr>
<td>Variable</td>
<td>BD patients (no = 42) according to vitamin D level</td>
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<tr>
<td>----------</td>
<td>-------------------------------------------------</td>
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<tr>
<td></td>
<td>Sufficient (&gt;30 ng/ml) (no = 17)</td>
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<td>Age (years)</td>
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<tr>
<td>Disease duration (years)</td>
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<td>Current smoker no (%)</td>
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<tr>
<td>Current steroid no (%)</td>
<td>1 (5.9)</td>
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<tr>
<td>Current Colchicine no (%)</td>
<td>13 (76.5)</td>
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<tr>
<td>BDCAF</td>
<td>3.1 ± 2.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD unless otherwise specified.

BDCAF: Behcêt’s Disease Current Activity Form.

* Significant at $p < 0.05$ $p > 0.05 = $ non significant.

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<thead>
<tr>
<th>Table 6</th>
<th>Correlations between vitamin D serum levels and variables in BD patients.</th>
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<tr>
<td>Variable</td>
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<tr>
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<tr>
<td>Disease duration (years)</td>
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<tr>
<td>BDCAF</td>
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<tr>
<td>ESR (mm/1st hour)</td>
<td>$-0.59$</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>$-0.53$</td>
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</table>

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, BDCAF: Behcêt’s Disease Current Activity Form.

* Significant at $p < 0.05$ $p > 0.05 = $ non significant.

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<th>Table 7</th>
<th>Multivariate regression analysis in BD patients.</th>
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<tr>
<td>Beta</td>
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<tr>
<td>Disease duration (years)</td>
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<td>Colchicine therapy</td>
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<td>ESR (mm/1st hour)</td>
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<td>CRP (mg/L)</td>
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<tr>
<td>BDCAF</td>
<td>0.17</td>
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</table>

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, BDCAF: Behcêt’s Disease Current Activity Form.

* = significant at $p < 0.05$ $p > 0.05 = $ non significant.

Figure 2 Correlations of serum vitamin D levels with patients’ Behcêt’s Disease Current Activity Form (BDCAF).
there was no correlation between vitamin D levels and ESR or CRP levels in BD patients. Timms et al. [48], in their study on healthy adults revealed that CRP levels were decreased by 23% in hypovitaminosis D patients who received approximately 547 IU of vitamin D daily for 2.5 years and suggested a dose-dependent anti-inflammatory effect of vitamin D.

The results of this study exhibited significant negative correlations of vitamin D serum levels with BDCF (p = 0.003). Our results go hand in hand with those of Do et al. [30] who 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 disturbance in the inflammatory condition. In the same context, Hamzaoui and his colleagues [49] found that in active BD patients, there were significantly inverse correlations between vitamin D levels and both CRP and ESR expression, indicating that disease activity is associated with lower vitamin D serum levels while, no correlations were observed in healthy controls and inactive BD patients. They also observed a significant and inverse correlation between vitamin D of active BD patients and age.

Dusso et al. [50] reported that a low vitamin D level is possibly an indicator for the inflammatory process as Inflammation itself potentially enhances vitamin D catabolism.

In multivariate regression analysis, age (p = 0.02), colchicine therapy, (0.008), ESR (0.02) and CRP (0.03) were found to be independent effectors of vitamin D levels.

Karatay and his associates [47], in multivariate regression analysis, stated that smoking, alcohol intake, and use of colchicine were the main predictors of vitamin D levels, although the effects of colchicine on vitamin D levels have not yet been fully defined.

In conclusion, this study demonstrated that serum levels of vitamin D were significantly lower in BD patients compared to controls. Associations were found between vitamin D levels and age, disease activity as well as ESR and CRP in BD patients. Low vitamin D may predispose those patients to active disease, especially in older subjects. More studies are required to determine whether treatment with vitamin D would have beneficial effects on patients with BD.

Conflict of interest

All the authors responsible for this work declare no conflict of interest.

Acknowledgment

We thank Prof Dr Samia Abdelmoneim for her unbounded support and revision of this work.

References

Vitamin D levels in patients with Behçet’s disease: Significance and impact on disease measures


