Vitamin D modulates peripheral immunity in patients with Behçet's disease

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SUMMARY

Background. *There is little knowledge about clinical and immunological variables associated with vitamin D insufficiency in inflammatory diseases.*

Objective. *We sought to investigate disease variables associated with vitamin D levels in patients with Behçet's disease (BD) and its interaction with inflammatory responses.*

Methods. *One hundred and sixty BD patients (102 patients in active stage) were enrolled in a study assessing the relationship between serum vitamin D concentrations and disease activity. As control diseases we studied 22 Rheumatoid arthritis (RA) and 30 multiple sclerosis (MS) patients. Serum concentrations of vitamin D were assayed with a radioimmunoassay kit. To assess the correlation between inflammatory mediators, immune cell expression and vitamin D, 20 active BD patients and 18 healthy controls were investigated: T-cell subsets and Treg cells were quantified by flow cytometry. Th1/Th2 ratio and Th17 were studied by intracytoplasmic cytokines expression (IFN-γ, IL-4, IL-10 and IL-17).*

Results. *Decreased levels of vitamin D were observed in active BD patients compared to patients in the inactive stage and to healthy controls (p=0.0246; p=0.0001). A low significant difference was observed between inactive BD and healthy controls (p=0.004). Active BD expressed higher vitamin D levels than RA (p=0.007) and MS (p=0.044) patients (p=0.0238). In active BD, vitamin D levels were correlated with CRP and ESR. Serum levels of vitamin D correlated positively with the number of Treg cells (r=0.640; p=0.0024). The IFN-γ/IL-4 ratio (Th1/Th2) was inversely correlated with serum 25(OH)D levels (r=-0.599; p=0.0053).*

Conclusions. *Active BD was associated with lower serum Vitamin D levels. Our results showed that low levels of vitamin D were associated with a decrease in Treg cells and a skewing of the Th1/Th2 balance towards Th1. These findings suggest that vitamin D is an important promoter of T cell regulation in vivo in BD patients. As suggested in other inflammatory/autoimmune diseases, vitamin D may modulate inflammatory mediators.*

Introduction

Behçet's disease (BD) is a chronic relapsing systemic inflammatory disease characterised by the presence of orogenital ulcers, cutaneous manifestations, and uveitis. The disease can also lead to vascular complications such as arterial and venous thrombosis, central nervous system vasculitis, arthritis, and gastrointestinal involvement (1). The etiology of Behçet's disease is not fully understood. Autoimmunity, genetic and environmental factors are thought to play a part in the pathogenesis of BD (2). $CD4^+$. T-lymphocytes seem to be the major cell-type in inflammatory infiltrates, and increased concentrations of tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) have been described (3, 4). Treatment remains insufficient and relies on non-specific immunosuppressive medications, with significant side effects.

There is an increasing interest in the role of vitamin D as a potential treatment for a number of disparate diseases. In addition to its role in calcium homeostasis, vitamin D has a plethora of effects inclu**d**ing immunomo**d**ulation, pleiotropic effects an**d** mo**d**ulating propensity to infection (5). Vitamin D binding protein (DBP) binds the vitamin D metabolites with high affinity (6). Among the cytokines, IL-6 may increase the expression of its mRNA, whereas TGF-β decreases its mRNA expression (7). Clinically, the reduced intake of vitamin D increases the prevalence of certain autoimmune/inflammatory diseases, such as multiple sclerosis, RA, SLE and inflamma-

tory bowel diseases (8). Serum vitamin D has been shown to possess important immunoregulatory functions and is essential in the regulation of autoimmune/inflammatory processes. In RA and MS patients $1,25(OH)_2D_3$ treatment *in vivo* induces a reduction in the autoimmune Th1 response targeting dendritic cells, $CD4^+$ T cells and regulatory T cells, associated with an amelioration of symptoms (9-10).

The aim of the present study was to assess vitamin D levels in patients with BD in correlation to clinical findings and to immunological parameters.

Table I. Baseline demographics and clinical characteristics of patients with Behçet's disease

Clinical characteristics	Patients (n=102)	Percentage (%)
Oral ulcer	100	98
Genital ulcer	98	96
Erythema nodosum	46	45
Ocular symptoms	52	50.9
Thrombophelibitis	21	20.58
Arthritis	34	33.3
Neurologic involvement	18	17.64
Pulmonary manifestation	22	21.56
The skin pathergy test (+)	84	82.35
The skin pathergy test (-)	18	17.64
HLA-B_{51}(+)	32	31.37
HLA-B_{51}(-)	70	68.62

Patients and methods

Patients

The patients' group consisted of 160 BD patients (active BD: 102, inactive BD: 58). Active BD patients fulfilling the International Study Group (ISG) criteria (11) were recruited from the internal medicine department (La Rabta), the neurological department (La Rabta) and the respiratory diseases department (Ariana). There were 40 females and 62 males. The mean age was 34.70±8.65 years (range: 18–49 years). Patients with inactive BD (n=58; 42 men, 16 women; mean age: 33.79±6.25 years; range: 20–42 years) did not received corticosteroid within 3 weeks. All active BD patients received corticosteroid treatment at the dose of 3 to 5mg/day (Group I), except for 20 patients (Group II) who received higher doses ranging from 10 to 50mg/day. This study was approved by the Institutional Review Board.

Clinical characteristics of BD patients are summarised in Table I. Mean disease duration was 9.45±5.36yrs. Most of the patients (98%) in the active BD group had oral aphthæ, 96% had genital ulcers and 50% had ocular symptoms. Only 31.37% were HLA-$B51^+$. Behcet's disease activity index (BDAI) was also performed on our patients according to the method presented by Bhakta *et al*. (12) and Lawton (13). Patients who had no symptoms related to BD in a four-week period, or less than two symptoms with a healing process and an overall wellbeing status, were grouped in inactive BD. ESR (mm/h) and CRP (mg/L) expressed as median (range) values in active BD patients were significantly increased in active BD patients (ESR: 35.76 (2–90); CRP: 107.5(3–260)) compared to healthy controls (ESR: 6(2–17); CRP: 3;2(3–9); $p<0.0001$). ESR and CRP values in inactive BD patients tended to be similar to those observed in healthy controls and were significantly different from active BD patients (ESR: 13.81 (4–35); CRP: 13.81 (4–35); $p=0.0001$). Patients and controls all lived in the northern part of Tunisia (latitude 39°N, longitude 9°E). The Tunisian population results from an important intermingling of different populations throughout history, mainly composed by Berbers, Arabs, Turkish and southern Europeans (14). None of the patients or controls enrolled in this study had ongoing infections, either viral or bacterial. No subjects received vitamin A, D or E supplementation during the study period and within 10 months before enrolment. None of the patients or controls had fat malabsoption due to liver, gallbladder, pancreatic diseases or coeliac disease.

Thirty MS and 22 RA patients acted as controls of the diseases. Informed consent was obtained from patients prior to enrolling them into the study. RA patients (16 men and 6 women; aged from 35–47 years; mean age: 39 years) were diagnosed by the 1987 revised classification criteria of the American College of Rheumatology (15). MS diagnosis (20 men, 10 women; ages: 32–46 years; mean age: 37 years) was established according to Poser's criteria (16). A cohort of age- and sex-matched 50 healthy individuals served as the healthy control (35 men and 15 women; aged from 21–46 years; mean age: 33.9±7.54 years). None of the controls enrolled in this study had ongoing infections, either viral or bacterial.

Determination of serum vitamin D levels

Blood withdrawal was performed in the period from October 2009 (autumn) until March 2010 (winter). The cells for the cellular assays and the serum for vitamin D measurement were retrieved at the same time, and cellular assays were performed on the day of blood collection. The collected serum was immediately shielded from direct light and stored at -20°C. At the end of the study, all samples were analysed simultaneously. Serum concentrations of 25(OH)D were assayed with a radioimmunoassay kit (Dia-Sorin, Stillwater, MN, USA). Following extraction of 25(OH)D using a donkey anti-goat precipitating complex, the treated sample was assayed according to the equilibrium radioimmunoassay procedure. The reference range for the assay was 9.0–37.6ng/ml. The determination of serum vitamin D levels included active and remission BD patients and 50 healthy volunteers.

Lymphocytes subsets T cell phenotyping

Lymphocytes were isolated from peripheral blood drawn from 20 active BD patients and 18 healthy controls as previously described (4). PBMC were isolated by Ficoll Hypaque gradient centrifugation (Histopaque; Sigma Aldrich, Zwijndrecht, The Netherlands). $CD4^+$T cells were selectively isolated with Ro-

setteSep (Stem Cell Technologies, Grenoble, France). The method for intracellular staining of $CD4^+$ T-cell subsets has been described in detail previously (4, 17). The following monoclonal antibodies were used: FITC-labelled anti-IFN-γ, PE-labelled anti-IL-4, PE-conjugated anti-IL-10 (all from BD Biosciences) or PE-labelled anti-IL-17 (R&D Systems, Minneapolis, MN, USA). Based on intracytoplasmic staining, the phenotypes within $CD4^+$ cells were determined as follows: Th1 cells, $CD4^+IFN^+IL\text{-}4^-$; Th2 cells, $CD4^+$IFN-IL-4^+; Tr1 cells, $CD4^+IL10^+$; and Th17 cells, $CD4^+IL17^+$. Cell surface (CD4, CD25) and intracellular (Foxp3) staining was carried out on freshly isolated PBMCs from heparinised blood as we have recently reported (17, 18). Lymphocytes were gated on the basis of their forward and side scatter properties by using FACSCalibur flow cytometry (Becton Dickinson, San Jose, CA, USA). Data were analysed using CellQuest software (Becton Dickinson). The following reagents were used: Ficoll and CD4-FITC monoclonal antibody (Sigma Aldrich, St Louis, MO, USA), CD25-PC5 (Immunotech), Foxp3-PE, clone: PCH101 (eBioscience) and intracellular staining kit (eBioscience).

CRP and ESR analyses

CRP was measured by a highly sensitive turbidimetric method using conventional biochemical automatic analyser. The upper limit of the normal range was 5mg/L. The ESR was determined by the classic Westergren method.

Statistical analysis

Statistical analysis was conducted with Statistical Package for Social Sciences version 15.0 software (SPSS inc., Chicago IL, USA) and figures were constructed with GraphPad Prism 5 software (GraphPad Software inc., La Jolla CA, USA). For the continuous variables, the median value and corresponding range (min–max) are provided. When normally distributed (Shapiro-Wilk test $p>0.05$), a linear relationship between two continuous variables was tested with the Pearson correlation coefficient (Pearson R). Abnormally distributed variables were Log-transformed. In case of persistent abnormal distribution, the Spearman correlation coefficient (Spearman R) was determined. Univariate relationships between Vitamin D levels and patients' demographic and corticosteroid treatment were determined using the Spearman rank correlation coefficient when the variables were continuous and the Wilcoxon test with ±2 approximation when they were categorical. These tests were chosen because of the nature of the retrospective convenience sample, the variability in sample size among variables, and the non normal distribution of variables. A p-value <0.05 was considered statistically significant.

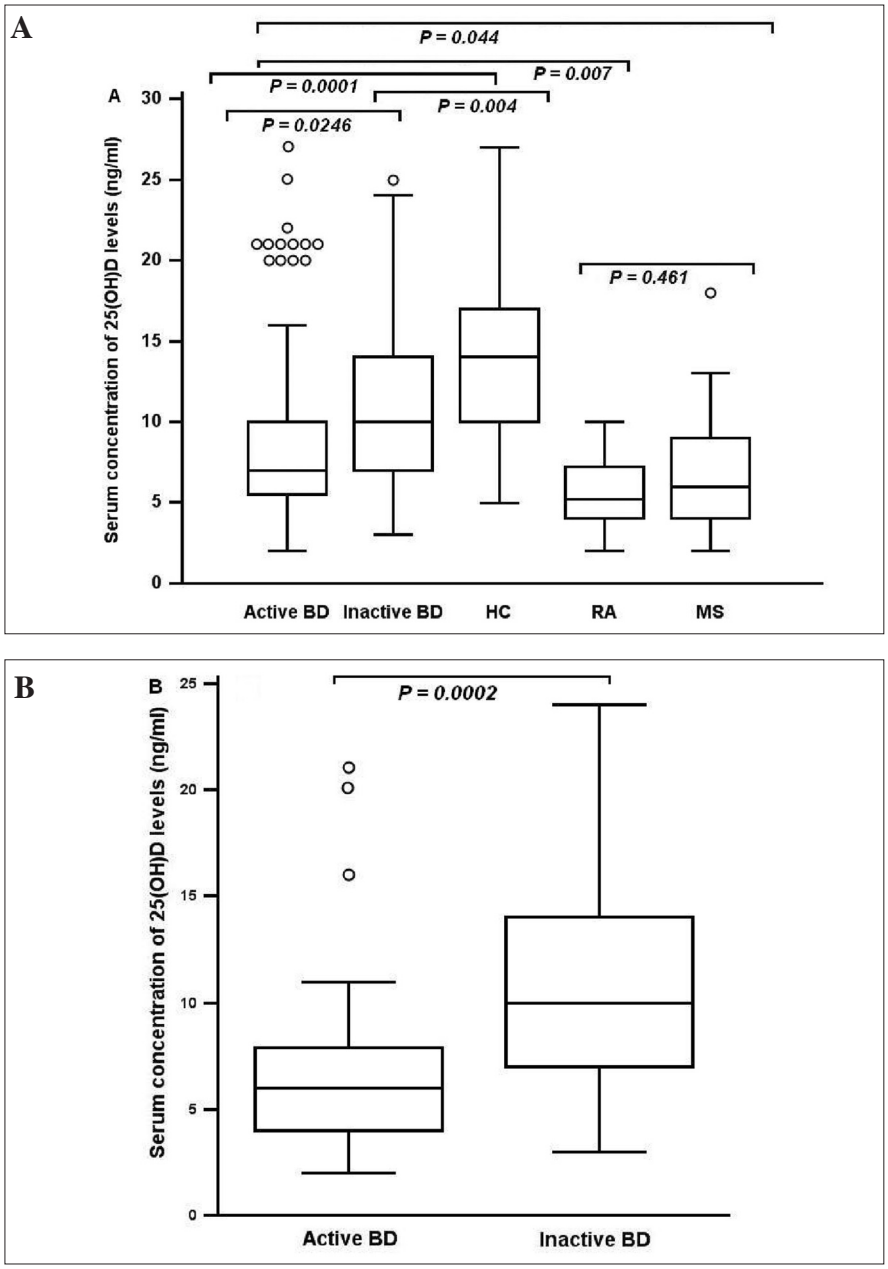

Fig. 1. Serum concentrations of 1,25(OH)D levels in Behçet's disease (BD). **A**: Box plot indicating serum 25(OH)D levels in active BD (n=102) patients (mean±SD: 7.431±3.28 ng/ml), in patients with inactive BD (n=58) (11.086±5.17ng/ml) and in healthy controls (n=50) (14.03±5.2ng/ml). Control diseases: MS (n=30) and RA (n=22) patients expressed lower levels than HC. **B**: Comparative analysis of 1,25(OH)D levels in serum taken from 40 patients at two different time points; when their BD was active, and when their BD was inactive. Statistical significance was evaluated by the Wilcoxon test. The medians are indicated by a line inside each box, the 25th and 75th percentiles by the box limits, the lower and upper error bars represent the 10th and 90th percentiles, respectively.

Results

Vitamin D level in Behçet's disease

We found lower serum vitamin D levels in active BD patients (mean±SD: 9.031±5.679 ng/ml; range: 2–227) compared to inactive BD patients (mean±SD: 11.08±5.17ng/ml; range: 3–25; p=0.0246) and healthy controls (14.03±5.20ng/ml; range: 5–27; p=0.0001) (Fig. 1A). A significant difference was observed between inactive BD and healthy controls (p=0.004). Vitamin D levels found in active BD patients were significantly different from values observed in RA patients (5.65±2.20ng/ml; range: 2–10; p=0.007). Differences between active BD and MS patients (6.80±3.63ng/ml; range: 2–18; p=0.044) were slightly different (Fig. 1A). No difference was found between MS and RA patients (p=0.1940).

Forty patients with BD were studied successively during active and inactive disease stages. Significant changes in their vitamin D levels were observed between active (6.91±4.15) and inactive stages (10.70±4.65; p=0.0002) (Fig.1B).

In a second step, patients were studied according to their status HLA-B51$^+$ or HLA-B51$^-$. Serum vitamin D levels in HLA B51$^+$ active BD patients(6.865±2.73ng/ml) were not significantly different from levels observed in HLA B51$^-$ active BD patients (7.52±3.45ng/ml; p=0.346). All active BD patients received corticosteroid treatment. According to corticosteroid treatment doses Group II (>5 mg/day) expressed lower vitamin D levels (3.92±1.38ng/ml) than Group I (3 to 5mg/day) (8.145±3.02ng/ml; p=0.0001).

The levels of vitamin D were correlated to the age of active BD patients (Fig. 2). A significant negative correlation was observed between the age of active BD patients and their respective vitamin D value (r=-0.298; p=0.0023) (Fig. 2). No correlation was found either in inactive BD patients (r=0.202; p=0.128) or in healthy controls (r=0.132; p=0.241). Patients with active BD (BD=102) and healthy controls (HC=50) were divided in two groups A and B according to their age and were compared for their vitamin D levels (BD_A; BD_B; HC_A; HC_B). Group A was composed of individuals aged from 18 to 30 years (BD_A or HC_A) and group B was composed of individuals aged from 31 to 49 years (BD_B or HC_B). A significant difference was observed in healthy controls (HC_A: 18.19±8.69ng/ml; HC_B: 13.87±4.83ng/ml; p=0.028). However, no significant difference was observed in active BD patients (BD_A: 10.22±4.58ng/ml; BD_B: 8.42±6.00ng/ml; p=0.124). No significant difference in vitamin D according to age was observed in MS and RA patients.

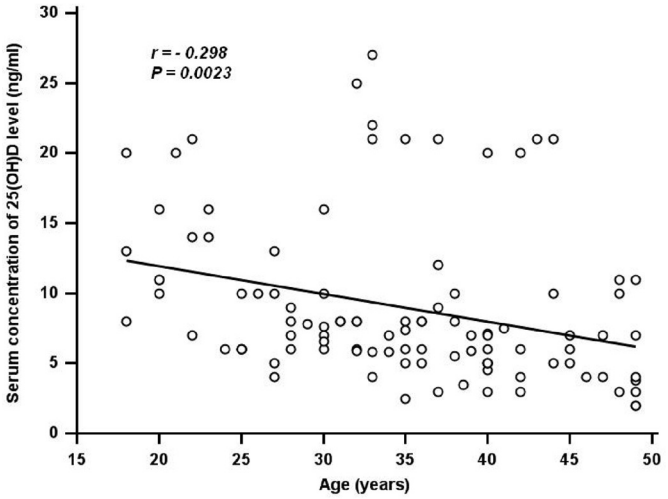

Fig. 2. Correlation between vitamin D levels and the age of active BD patients (n=102) using Pearson's correlation coefficient. Significant inverse correlation was observed between active BD patients and the level of vitamin D (r=-0.298; p=0.0023).

There were no significant differences of vitamin D levels according to gender in BD patients (Males: 9.95±5.40; Females: 9.60±5.75, p=0.687), in MS (Males: 6.455±3.812; Females: 7.50±3.341, p=0.707), in RA patients (Males: 5.62±2.43; Females: 5.71±1.55, p=0.933) and in healthy controls (Males: 13.157±5.13; Females: 16.06±4.92, p=0.867).

Vitamin D concentrations and clinical parameters

This analysis was performed on active BD patients (102 patients). Levels of vitamin D resulted significantly lower in active BD patients with pulmonary involvement (n=22) (5.36±1.59ng/ml) or neurological manifestations (n=18) (4.85±2.29ng/ml) compared to other patients (10.207±5.96; 10.48±3.48; p=0.0033 and p=0.0027).

Serum vitamin D levels were significantly and negatively correlated with CRP (r=-0.363; p=0.0002) (Fig. 3A) and ESR (Fig. 3B) in active BD patients (r=-0.256; p=0.0092). No correlations were observed in healthy controls and inactive BD patients.

The Th1/Th2 balance correlates negatively with vitamin D levels

Twenty active BD patients were studied simultaneously for their serum vitamin D level and for their CD4$^+$T cells in the peripheral blood. CD4$^+$T cells were classified as Th1, Th2 or Th17 by assessing their intracellular cytokine profile (IFN-γ, IL-4 and IL-17). We also assessed the percentage of CD4$^+$T cells producing the regulatory cytokine IL-10, known as an anti-inflammatory cytokine.Vitamin D level in this group of patients was 8.37±5.36ng/ml (range: 3–21ng/ml). The median percentage of IFN-γ $^+$cells within the CD4$^+$T cell compartment was (10.10%±4.98%; range: 3%–20%), of IL-4$^+$cells (11.30%±3.27%; range: 7%–218%), of IL-17$^+$cells (0.86%±0.58%; range: 0.3–2.5), and of IL-10$^+$cells (1.26%±0.71%; range: 0.38–2.56). A significant positive correlation between serum 25(OH)D levels and the individual percentages of T helper cell subsets was found (r=0.466; p=0.0382), in contrast 25(OH)D was negatively correlated with the percentage of IFN-γ$^+$CD4$^+$T cells (r=-0.552; p=0.0116). The balance between Th1 and Th2 in BD is used to indicate inflammatory status of the T cell compartment as we have recently reported (4). This balance is described by the ratio of IFN-γ$^+$and IL-4$^+$CD4$^+$T cells. The

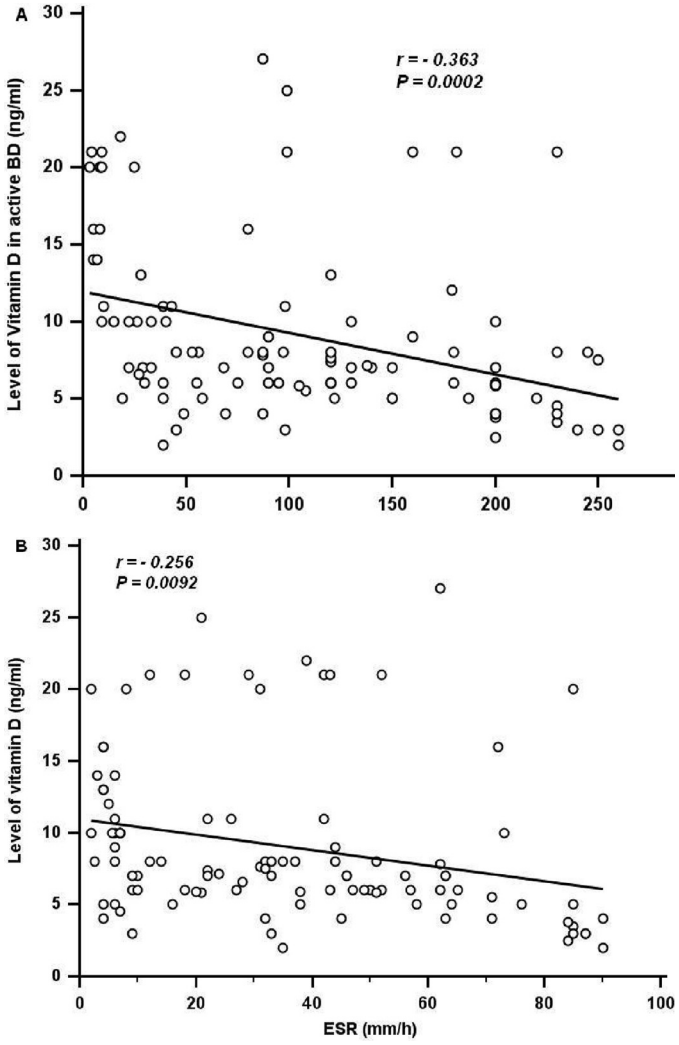

Fig. 3. Correlation between vitamin D levels and biological parameters CRP and ESR in active BD patients (n=102) using Pearson's correlation coefficient. **A**: Significant (negative) correlation was observed between the level vitamin D and C-reactive protein (CRP) measurements (r=-0.363; p=0.0002). **B**: Significant (negative) correlation was observed between the level vitamin D and erythrocyte sedimentation rate (ESR) (r=-0.256; p=0.0092).

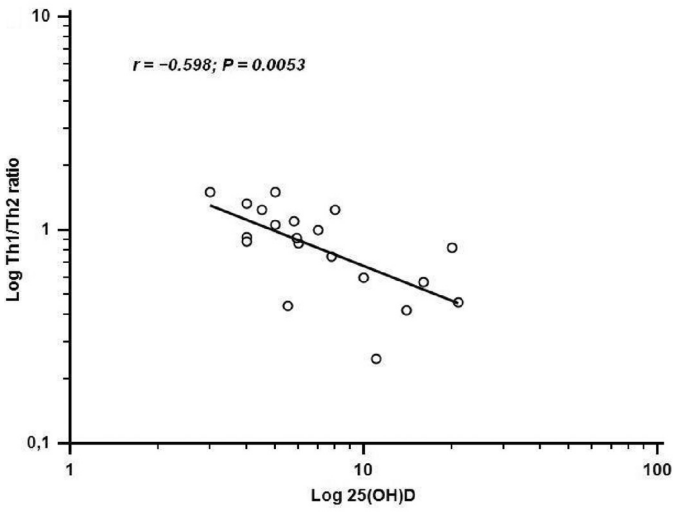

Fig. 4. Correlation of T helper cell subsets with serum 25(OH)D levels. The Pearson correlation coefficient and corresponding regression line are shown (r=-0.598; p=0.0053).

IFNγ/IL-4 ratio correlated negatively with serum 25(OH)D levels (r= -0.599; p=0.0053) (Fig. 4). IL-17$^+$ cells were less significantly correlated with vitamin D levels (r= -0.462; p=0.0403).

Treg cells correlate with serum vitamin D levels

Treg cells were evaluated in the peripheral circulation of 20 active BD patients as we have recently reported (17-18). Regulatory cells within the CD4$^+$T cell compartment were defined as CD25^{high}FoxP3$^+$cells. The median percentage of CD25$^+$FoxP3$^+$ Treg in the CD4$^+$ T cell compartment was (mean±SD: 6.7%±1.4%; range: 5.8–210.7%). Serum levels of 25(OH)D were positively correlated with the percentage of Treg cells (r=0.640; p=0.0024) (Fig. 5A). A significant positive correlation was also observed with IL-10 level (r=0.808; p=0.0001) (Fig. 5B).

Discussion

Contrasting levels of vitamin D have been found between active and inactive BD patients. Likewise, we found that 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 in active BD patients. Epidemiological studies suggest that adequate vitamin D levels decrease the risk of developing autoimmune diseases such as multiple sclerosis, inflammatory bowel disease and RA (19-21). Preventive treatment with vitamin D of individuals considered at high risk of developing autoimmune diseases has been proposed (22). Our results in BD were in accord of those reported by Do *et al*. who found that the serum vitamin D levels were inversely correlated with the serum CRP and the ESR levels in BD (23). The serum vitamin D concentrations were lower in patients with lung involvement and neurological manifestation in comparison with active BD patients without these clinical manifestations, supporting a connection between vitamin D levels and disease phenotype. Patients with active BD receiving high doses of corticosteroids exhibited the lowest vitamin D levels in their serum.

In our report, we observed decreased levels of vitamin D in active BD patients in the same way as in RA and MS patients. Low vitamin D levels were reported in patients with autoimmune diseases, including rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (24-26).

We observed significant and inverse correlation between vitamin D of active BD patients and their respective age, in

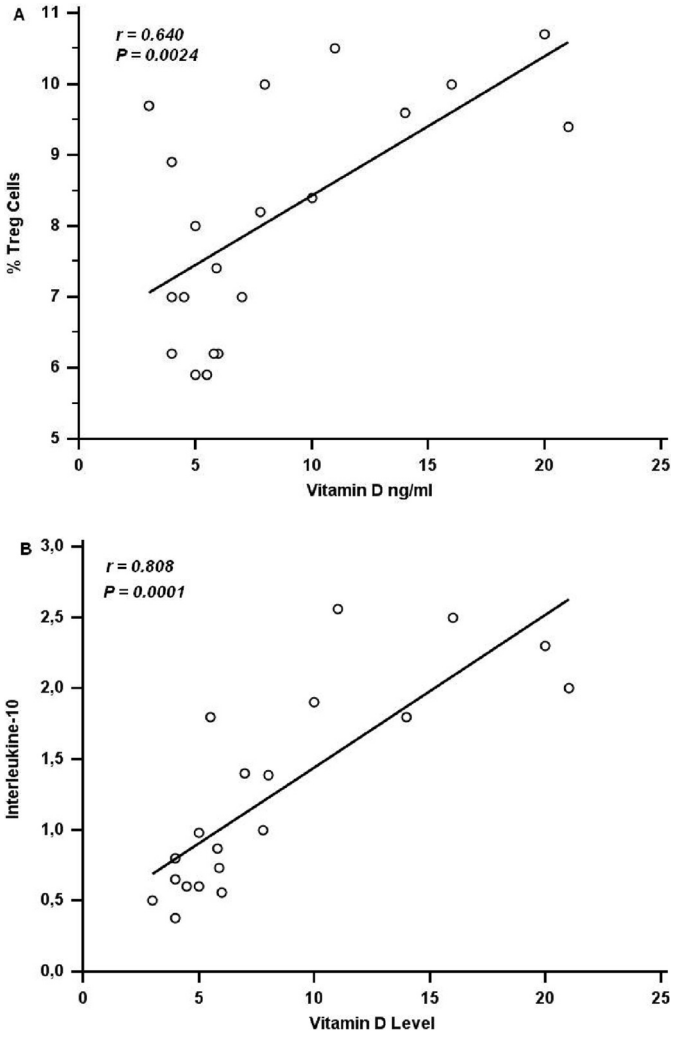

Fig. 5. Correlation analysis between levels of vitamin D and Treg cells and IL-10 using Pearson's correlation coefficient in active Behçet's disease (n=20). A *p* value <0.05 was considered as statistically significant. **A**: Correlation of the percentage of circulating Treg cells with serum 25(OH)D levels. The Pearson correlation coefficient and corresponding regression line are shown. **B**: Correlation between vitamin D levels and IL-10 levels.

contrast with Do *et al*. (23). Recently, Searing *et al*. (27) reported that the age of asthmatics was inversely correlated with serum vitamin D levels. The calcium status of the host may influence the effect of vitamin D on immunity as reported by Yu *et al*. (28). This could explain the inverse correlation observed in aged BD patients.

This is the first study to investigate the correlation between vitamin D status and the composition of the T cell compartment *in vivo* in active BD patients. Treg cells in the peripheral blood of active BD patients were significantly correlated with vitamin D status. The decreasing Th1/Th2 ratio reflected a skewing of the IFN-γ/IL-4 -balance towards a more Th2 phenotype in patients with higher serum 25(OH)D levels. Altogether, this data indicates that high serum 25(OH)D levels are associated with a less inflammatory and better regulated T cell compartment in active BD patients. The data obtained reflect the status of the T cell compartment at the moment of blood collection in strict correlation with the inflammatory process in BD. The fluctuations of immunological parameters associated to the fluctuation of serum 25(OH)D levels were probably specific to disease inflammation.

Several studies suggested that vitamin D deficiency could lead to immune malfunctioning (9, 10, 29. 30). Although the exact mechanisms of lower vitamin D levels in chronic inflammatory states are not yet elucidated in BD, the role of vitamin D as an immunomodulator is well known. T and B lymphocytes, macrophages and dendritic cells express Vitamin D receptors (VDR). The cells mostly affected by vitamin D are probably dendritic cells, modulating their maturational state (31).

We found a negative correlation between serum 25(OH)D levels and the IFN-γ/IL-4 ratio. This ratio is commonly used to describe the balance in the immune system between pro-inflammatory IFN-γ^+Th1 cells and anti-inflammatory IL-4^+Th2 cells(4). Our data suggest that in patients with relatively high 25(OH)D-levels, the balance between Th1 and Th2 is skewed in the direction of Th2. Interestingly, as reported by Correales *et al*. (32) and Smolders *et al*. (33) *in vitro* research also suggested that vitamin D skews the T cell compartment from a Th1 towards a Th2 phenotype. Therefore, high 25(OH)D levels appear to be associated with a less-pro-inflammatory T-cell compartment.

Disturbances in vitamin homoeostasis may contribute to the inflammatory process in active BD. Th1, Th2 cells and regulatory T cells have been shown to express the vitamin D receptor and to be vitamin D targets (8; 34-35). Recently Do *et al*. (23) reported higher expressions of TLR2 and TLR4 with a trend towards lower 25(OH)D levels in active BD patients. They also demonstrated the effect of vitamin D on innate immunity-mediated inflammation, enhancing the antimicrobial properties of immune cells such as monocytes and macrophages (36-37). In addition, vitamin D directly affects T cell responses, by inhibiting the production of Th1 cytokines (IL-2 and IFN-γ), Th17 cytokines (IL-17 and IL-21), and by stimulating Th2 cytokine production (IL-4). In BD, vitamin D could be considered as an important mediator which fluctuation is correlated to the inflammatory state of the disease. The development of certain immune cells requires the expression of the VDR both intrinsically and extrinsically as reported by Yu *et al*. (28) and Maruotti *et al*. (38). The importance of the Fok-I VDR polymorphism for vitamin D metabolism in BD is under investigation (data not shown).

The alteration of innate immune systems could critically be involved in the pathogenesis of BD (39-41). However, it is not clear what kind of stimuli and mechanisms are responsible for the *in vivo* activation of the immune system of BD patients. In a previous study, we

have reported the functionality *in vitro* of $CD4^+CD25^{high}$ Tregs in BD patients (17, 18). We have reported that active BD patients have higher Treg cells (17, 18). In the present study we found a significant correlation between vitamin D levels and $CD4^+CD25^{high}$ $FoxP3^+$ cells, as vitamin D by modulating dendritic cells, favour suppressive activity of Tregs as reported by Szeles *et al*. (42) by inhibiting the production of IL-12 and IL-23, and enhancing the release of IL-10 and MIP-3α (43-45). IL-10 was significantly correlated with vitamin D level in active BD. This result should indicate that vitamin D levels, IL-10 and Treg cells operate intimately to abrogate inflammation *in vivo*. This result together with the inverse correlation between IFN-γ/IL-4 ratio and vitamin D indicates that the increase in vitamin D level is associated with improvement of the patients.

During the BD active phase, T cells and particularly $CD4^+$ lymphocytes are intensively stimulated (39-41) and switched from naïve to memory CD4+T (41-42). Our explanation for the decreased vitamin D in the active stage is that CD4 cells consumed intrinsic vitamin D levels during their activation. It has been reported that quiescent $CD4^+$T cells express VDRs at low concentrations, which increase five-fold after their activation (36). Addition of 1,25(OH)2D3 leads to decreased secretion of IL-2 and IFN-γ by CD4 T cells and promotes IL-5 and IL-10 production, which further shifts the T cell response towards Th2 dominance (37). Importantly, immune cells are able to activate vitamin D locally, arguing for an autocrine or paracrine role for this hormone within the immune system.

The fact that Th1, Th2 and Th17 were correlated to serum vitamin D implies the origin of vitamin D deficiency in BD. More studies are needed to clarify the mechanisms by which vitamin D regulates cellular immunity and whether there are any genetic factors modifying production of $1,25(OH)_2D_3$ and signalling through the vitamin D receptor. VDR polymorphisms fok I are under investigation in BD.

In summary, our study provides important information on vitamin D levels and active BD. We were able to show that vitamin D insufficiency in active BD patients is common and similar to that seen in the MS and RA patients. We found significant correlations between several inflammatory and immunological markers and vitamin D levels. These findings should be confirmed in a prospective way with the generation of an efficient multivariate model allowing further research about the use of vitamin D supplementation in patients with active BD.

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