Effect of vitamin D deficiency and replacement on endothelial functions in Behçet's disease

M. Can[1], M. Gunes[2], O.A. Haliloglu[3], G. Haklar[4], N. Inanç[1], D.G. Yavuz[2], H. Direskeneli[1]

[1]Department of Rheumatology, [2]Department of Endocrinology and Metabolism, [3]Department of Internal Medicine, [4]Department of Biochemistry, School of Medicine, Marmara University, Pendik Training and Research Hospital, Istanbul, Turkey.

Meryem Can, MD
Mutlu Gunes, MD
Ozlem Asmaz Haliloglu, MD
Goncagul Haklar, Professor
Nevsun Inanç Associate Professor
Dilek Gogas Yavuz, Professor
Haner Direskeneli, Professor

Please address correspondence to:
Meryem Can, MD,
Department of Rheumatology,
Pendik Training and Research Hospital,
Pendik, Istanbul, Turkey
E-mail: meryemkrk@gmail.com

Received on March 12, 2012; accepted in revised form on August 29, 2012.

Clin Exp Rheumatol 2012; 30 (Suppl. 72): S57-S61.

Key words: endothelial dysfunction, Behçet's disease, vitamin D3

Competing interests: none declared.

ABSTRACT

Objective. *Endothelial dysfunction is previously demonstrated in Behçet's disease (BD) and vitamin D is implicated to affect endothelial functions. We aimed to evaluate the status of serum 25(OH)Vit D3 levels and its association with disease activity, endothelial function and carotis intima media thickness (CIMT) in patients with BD.*

Methods. *Thirty-six BD (F/M: 22/14, mean age: 39.6 years) patients and 51 healthy controls (F/M: 28/23, mean age: 34.5 years) were studied. Rheumatoid arthritis (RA) (n=33) patients (F/M: 26/7, mean age: 50.82 years) were also enrolled, as a disease control group. Endothelial function was evaluated by brachial artery flow mediated dilatation (FMD) and CIMT with B-Mode ultrasound. The vitamin D-deficient BD patients received 1000 IU Vitamin D3 daily for 3 months.*

Results. *Less than 50 nmol/L levels of 25(OH) Vit D3 were present in 61.1% (n=22) of BD and 35.3% (n=18) of HC (serum 25(OH)Vit D3 levels: BD: 44.5 (9-112) vs HC: 56 (14-125) nmol/lt, p=0.01). CIMT and FMD were also significantly different between BD and HC [0.56 (0.35-9.26) vs. 0.39 (0-0.52) and 5.20 (0.56-30.58) vs. 9.04 (-6.9-34.17), p=0.001 and p=0.02, respectively]. However, no correlation was observed between 25(OH)VitD3 levels and CIMT or FMD (r=0.6, p=0.7 and r=0.03, p=0.8, respectively) at baseline. CIMT measurements improved after replacement therapy (0.56 vs. 0.49, p=0.02), FMD measurements also improved, but not reaching statistical significance (5.2 vs. 8.28, p=0.06).*

Conclusion. *A high presence of vitamin D deficiency was observed in BD patients from Turkey and replacement of vitamin D had favourable effects on endothelial function.*

Introduction

Behçet's disease (BD) is a chronic, multisystemic, inflammatory disease characterised by recurrent attacks of mucocutaneous, ocular, musculoskeletal, vascular, central nervous system and gastrointestinal manifestations (1). Although still of unknown etiology, genetic factors such as HLA-B*51, cytokines IL-23R and IL-10 and intra-cellular signalling pathways such as UBAC2 are implicated. Both innate and adaptive immune activation has been demonstrated. Among pattern-recognition receptors, toll-like receptor-2 (TLR2) and TLR4 have been identified as signalling receptors activated by bacterial cell wall components and possibly have a major role in the innate-activation of BD (2).

It was previously reported that endothelial dysfunction (ED) is a marker of vascular involvement in any disease affecting the vascular structure. Ultrasound, a non-invasive imaging technique, utilises endothelium-dependent or flow-mediated dilatation (FMD) of the brachial artery and is accepted as the standart method to investigate endothelial dysfunction (3). Histopathological features of vascular-BD are mainly characterised by vasculitis, with prominent neutrophil and monocyte infiltration in the perivascular regions (4). Several studies have revealed indirect evidences of endothelial dysfunction in BD, such as increased von Willebrand factor, VEGF, MMPs and trombomodulin levels as well as coagulation and fibrinolytic pathway abnormalities(10). Immune-mediated vascular injury with increased expression of proinflammatory and T-helper type 1 (Th1) and Th17 cytokines, adhesion molecules and free oxygen radicals has been suggested as the main pathology underlying the ED (6). Recent studies have also shown that an increased carotid intima-media

thickness (CIMT) significantly correlated with endothelial cell dysfunction in BD.

Vitamin D is an environmental factor important in physiological immune functions and vitamin D deficiency might lead to immune malfunctioning (7-9). The prevalence of autoimmune diseases correlate with vitamin D levels and the reduced intake of vitamin D increases the prevalence of certain autoimmune-inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease (10). Vitamin D is recently shown to modulate endothelial functions (11). The effects of vitamin D on the endothelium might be due to the changes in paratyroid hormone, inflammation, oxidative stress, adipokines and energy metabolism. The immunomodulatory effect of 1,25 (OH) D3 through down-regulation of the expressions of TLR2 and TLR4 was demonstrated in human monocytes in an *in-vitro* model. In this study, enhanced expression of monocyte TLR2 and 4 are inversely correlated with serum 25 (OH) Vit D3 levels in BD patients (12).

With this background, we designed a prospective study with the following objectives: 1. to determine the prevalence of vitamin D deficiency in BD patients from Turkey; 2. to establish the association between vitamin D levels and disease activity; 3. to identify the association between vitamin D levels with endothelial function and carotid intima -media thickness and the impact of VitD replacement therapy.

Material and methods

Patients

Thirty-six BD (F/M: 22/14, mean age: 39.6 years) patients and 51 healthy controls (F/M: 28/23, mean age: 34.5 years) were studied between February-April 2010. All BD patients fullfilling the International Study Group Criteria (ISGC) were recruited from the Rheumatology outpatient clinic (13). On the day of vascular examination, patients with active BD were receiving colchine (n=11), azathioprine (n=3), corticosteroids (prednisone: 5mg/day, n=4) and one patient was receiving interferon-α therapy. Among the remaining patients, classified as having inactive disease (n=24), 9 were receiving corticosteroids (prednisone: 5 mg/day), 21 colchicine, 10 azathioprine and 1 patient was on interferon-α therapy. At the 3-month evaluation, the number of active patients (n=9) and steroid usage was not different (23/13 *vs*. 29/7, p=0.1), compared to baseline. Patients with RA (n=33, F/M: 26/7, mean age: 50.82 years) who were classified according to the 1987 revised ACR criteria were also studied, as a disease control group (14). Disease activity of RA patients were assessed by DAS28 score, ESR and CRP. Height, weight, body mass index (BMI) and blood pressures were noted.

Endothelial function was evaluated by brachial artery flow mediated dilatation (FMD) and CIMT was measured with B-Mode ultrasound. The vitamin D-deficient BD patients received 1000 IU vitamin D3 daily for 3 months. At the end of the third month of treatment, the subjects were re-evaluated for the same parameters (CIMT and FMD).

Measurement of biochemical parameters

Serum 25OH Vit D3 levels were measured with HPLC (Thermofinnigen,Germany). Serum 25OH Vit D3 levels below 50 nmol/lt were considered to be low. Concentration of 25OH Vit D3 20ng/ml (<50nmol/lt) are adopted as "deficient" and <30ng/ml (<75nmol/lt) as "insufficient". Intact PTH levels were measured with electrochemiluminence immunoassay method (ECLIA) (Roche Diagnostics GmbH, Indianapolis, USA) (normal range: 10–65pg/ml).

Endothelial function assessment

Endothelial function was evaluated by high-resolution Doppler ultrasonography examination of right brachial artery measuring FMD, as previously described (3). Measurements were taken in the morning after patients had a rest in a supine position for 20 minutes in a quite room. All evaluations were performed after a fasting of 8 hour, at a room temperature of 20–25ºC, by a single sonographer (MG) who was blinded to diagnosis and clinical records. All patients abstained from fatty meals and caffeine containing drinks for at least 12 hours and cigarette smoking for 2 days before testing. Endothelial function of female patients were assessed during the follicular phase of menstrual cycle. A linear array transducer with a frequency of 10 MHz was used to acquire images. Brachial artery evaluations was performed 2 cm above the right antecubital fossa. ECG was monitored continuously. After measuring the basal diameter and flow rate, arterial occlusion was created by cuff inflation to 250–300 mmHg for 5 minutes. After the cuff deflated, lumen diameter was noted 1 minutes later to assess FMD. FMD was then calculated as the percentage increase in diameter from baseline. Carotid artey intima-media thickness was also measured using the same ultrasound transducers as described previously. The common carotid arteries were scanned longitudinally. The bulb dilation serve as a landmark to indicate the border between distal common carotid artery and the carotid bulb. Images were obtained from the distal portion the common carotid artery, 1–2 cm proximal to the carotid bulb. The two bright echogenic lines in the arterial wall were identified as the intima and media lines.The intimal plus medial thickness was measured as the distance from the main edge of the first line to main edge of the second line.

Statistical analyses

All statistical calculations were performed with the SPSS (Statistical Package for Social Sciences) for Windows 15.0 software. Comparisons between the groups were made using the paired *t*-test and Mann-Whitney U-tests. The Pearson correlation with two-tailed probability values was used to estimate the strength of association between variables.

Results

No difference in terms of a history of hypertension, smoking and body mass index were present among the three study groups and none of the subjects were diabetic. All BD patients had oral ulcers. Twenty-eight patients (77.8%) had genital ulcers, 25 (69.4%) had skin lesions, 11 (30.6%) had vascular, 9 (25%) had ocular and 2 (5.6%) had

neurological involvement. Patergy test was positive in 23 (63.9%) patients. Twelve (33.3%) BD patients had active disease at baseline evaluation. Six patients had oral ulcers, 2 patients had arthritis and ocular involvement, one patient had vascular involvement and a genital ulcer. Mean DAS28-score was 2.9 (1.1) in RA patients. Median (min-max) ESR (mm/h) and CRP (mg/l) levels were 24 (7-67) mm/h and 7 (2-25) mg/L. Rheumatoid factor was present in 72.7% and anti-CCP in 48.5% of the study group. Both baseline and post treatment calcium, phosphorus and intact PTH levels were similar (Table I).

Table I. Laboratory results of biochemical parameters.

	Before treatment	After treatment	*p*-value
iPTH(pg/ml)	58.9 (23.4)	40.5 (21.2)	ns
Ca(mg/dl)	9.5 (0.3)	9.5 (0.4)	ns
P(mg/dl)	3.9 (0.5)	3.8 (0.5)	ns

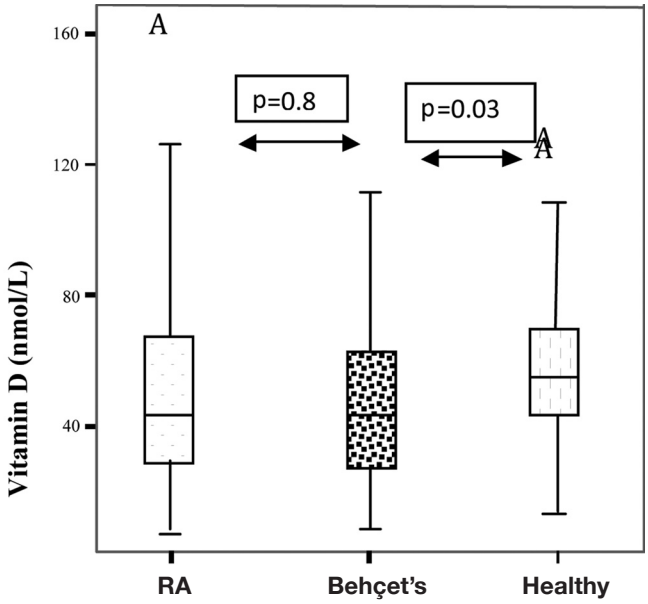

Fig. 1. Serum concentrations of 25(OH)Vit D3 in all groups. The medians are indicated by a line inside each box, the 25th and 75th percentiles by the box limits, the upper and lower error bars represent the minimum amd maximum .

Vitamin D levels and endothelial assessments

Less than 50 nmol/L levels of 25(OH) Vit D3 were present in 61.1% (n=22) of BD, 35.3% (n=18) of HC and %59.4 (n=19) of RA patients. A significant difference was observed between the levels of 25(OH) Vit D3 among BD and HC (BD: 44.5 (9–112) *vs*. HC: 56 (14-125) nmol/liter, *p*=0.03), but no difference was present between RA and BD [44 (8–162) *vs*. 44.5 (9-112), *p*=0.8] (Fig. 1). Levels of 25(OH)VitD3 were not significantly different between active and inactive BD patients [39.5 (14–65) *vs*. 53.8 (9–112) *p*=0.09].

FMD measurements for Behçet's disease patients were significantly lower compared to HC group at baseline [5.20 (0.56–30.58) *vs*. 9.04 (-6.9–34.17), *p*=0.02]. CIMT measurements at baseline were also significantly different between BD and HC [0.56 (0.35–9.26) *vs*. 0.39 (0–0.52), *p*=0,001]. FMD measurements were significantly different between active and inactive BD patients [11.5 (10.5) *vs*. 7.2 (6.03), *p*=0.04] but CIMT measurements were not different [0.35 (0.1) *vs*. 1.2 (2.4) *p*=0.1], respectively.

No correlation was observed between 25 (OH) VitD3 and CIMT or FMD (r=0.06, *p*=0.7 and r=0.03, *p*=0.8, respectively). Also no correlation was observed between 25 (OH) VitD3 and CIMT with disease activity in BD (r=2.28, *p*=0.09 and r=0.2, *p*=0.13, respectively) but a negatif correlation was found with FMD (r=-0.34, *p*=0.03).

No significant increase of 25(OH) VitD3 levels after replacement therapy [44.5 (9–112) *vs*. 47.5 (13–112) *p*=0.2] was observed after 3 months of therapy. However, after replacement therapy, CIMT measurements improved (1.04 *vs*. 0.50, *p*=0.001), FMD measurements also improved but not reaching statistical significance (6.3 *vs*. 7.8, *p*=0.4) (Fig. 2-3).

Discussion

In this study, we demonstrated a high presence of vitamin D deficiency in BD patients from Turkey. Although impaired vascular endothelial function did not correlate with vitamin D levels, replacement of vitamin D had favourable effects on endothelial function. These results suggest an immunomodulatory effect of vitamin D on endothelial inflammation in BD.

Current evidence suggests that vitamin D plays an important role in the modulation of immune system. Vitamin D regulates the balance between Th1 and Th2 cells. Basic studies have also shown inhibitory properties of vitamin D on Th1 immunity and autoantibody production. Epidemiological studies suggest that adequate vitamin D levels decrease the risk of developing autoimmune diseases such as inflammatory bowel diseases and RA(15). Preliminary data suggest an inverse relationship between serum 25(OH) Vit D3 levels and disease activity score (DAS28) in RA (16-17). In the same manner, although we observed decreased levels of serum 25(OH) Vit D3, a corrrelation with clinical parameters was not present. Welsh *et al*. showed that despite an inverse association between baseline measures of inflammatory status in RA and circulating 25(OH) Vit D3 levels, short-term TNF-α antagonist (adalimumab 40 mg/2 weeks apart for 16 weeks) treatment does not improve 25(OH) Vit D3 levels (18).

In a recent study, Hamzaoui *et al*. also showed that low levels of vitamin D were associated with a decrease in Treg cells and skewing of the Th1/Th2 balance towards Th1 in BD. Active BD was also associated with lower serum vitamin D levels in this study (19). However, Kandi *et al*., showed no significant difference of vitamin D levels between BD patients and controls, though mean

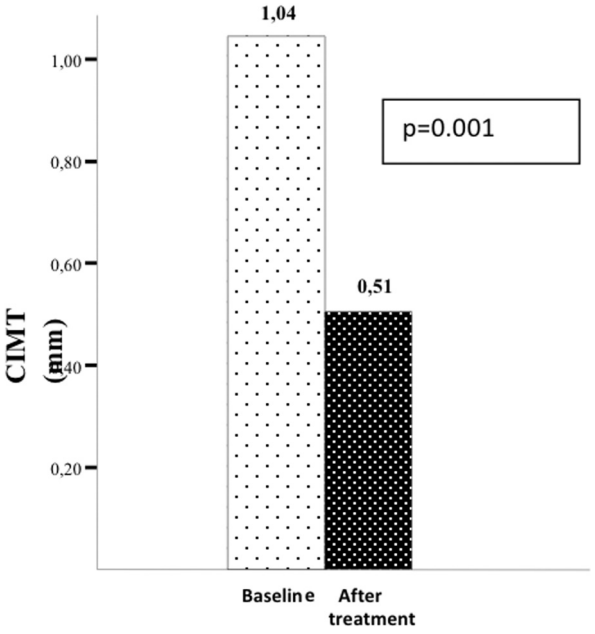

Fig. 2. In BD patients, CIMT values were significantly increased after treatment compared with baseline (p=0.001)

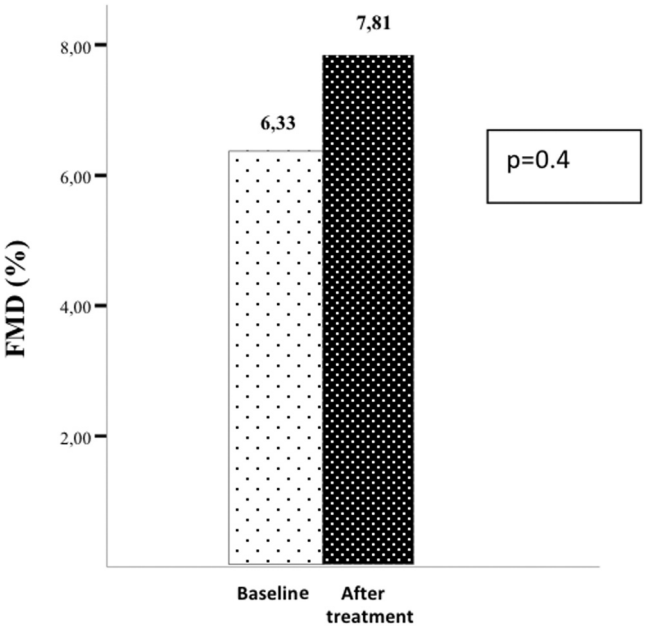

Fig. 3. In BD patients, FMD values improved after treatment compared with baseline (p=0.001).

values in BD patients was lower (20). Je Do *et al*. found that the monocytes of active BD patients showed higher expressions of TLR2 and TLR4 than those of controls, and serum 25(OH)D3 levels tended to be lower in active BD. Moreover, 25(OH)D3 levels inversely correlated with the expressions of TLR2, TLR4 and clinical parameters (12). We found a trend towards lower serum 25(OH)D3 levels in active BD, but not reaching a statistically significant level. These discrepant results might be explained with genetic factors, different disease activities among study populations (milder activity and immunomodulatory medications) and climate effects (conduction of studies in winter *vs*. summer time). However, most studies showed a trend towards lower serum 25(OH)D levels in active BD patients, suggesting that vitamin D deficiency might be a risk factor for disease activation and dysregulated inflammatory status. Serum vitamin D concentrations were lower in patients with lung involvement and neurological manifestation in one study, supporting also a connection between vitamin D and disease phenotype (21).

It was previously reported that endothelial dysfunction is a marker for vascular involvement in any disease affecting vascular structure including BD, which characteristically cause venous vasculitis (22, 23). The effects of vitamin D on endothelial function may be through direct or indirect mechanisms. Vitamin D receptors are present on endothelial cells and the role of vitamin D as an immunomodulator is well known. T/B lymphocytes, macrophages, and dendritic cells express vitamin D receptors (VDR). Stimulation through TLRs upregulate the expression of VDRs on the surface of macrophages. Similarly, *in situ* conversion of 25 OH D to the active form 1,25 OH D which has a costimulatory effect on lymphocytes activate adaptive immunity(15, 20, 24, 25). An enhanced expression of monocyte TLR2 and TLR4 which participate in innate immune activity, is reported in BD and are inversely correlated with serum 25 OH Vit D3 levels, suggesting that Vit D might participate in enhanced innate activity in BD (15).

In this study, we demostrated that carotid IMT was significantly higher in BD patients and improved after replacement therapy with VitD. Previous studies, similarly, demostrated high CIMT in BD patients (22, 23). Hong *et al*. also reported that increased arterial wall thickness was independently associated with the disease duration and the disease activity score (26). These results suggest that BD itself may lead to an increased CIMT, possibly due to inflammation. Antioxidant system is disturbed in BD and may be another reason for the development of ED besides the lipid abnormalities and inflammation which was reported by Chambers *et al*. Improvement of CIMT with VitD implies that long-term cardiovascular protection can be attained by replacing VitD deficiency in BD patients (6, 27).

Vascular endothelial growth factor (VEGF) is a potent angiogenetic factor and a marker for endothelial dysfunction. Hamzaoui *et al*. showed that CSF-VEGF and serum VEGF were detected at high levels in BD patients with neurological involvement. A significant correlation was observed between CSF $CD4^+$ cells and CSF-VEGF. These re-

sults indicate that VEGF may play a role in neuro-BD (28). Also in another study, they found that a positive correlation between MMP-9 and TIMP-1 levels in neuro-BD patients but not in NIND (noninflammatory neurological disease) or BD patients free from any CNS inflammation. MMP-9 has been shown to degrade components of the basal lamina and disrupt the blood-brain barrier and contribute to neuroinflammation (29). Pay *et al*. reported that MMP-9 can be used as an activity indicator for BD. The systemic expressions of MMP-2 and MMP-9 were strongly associated with vasculo-Behçet's disease, particularly aneurysmatic involvement (30). These angiogenetic factors might also influence endothelial dysfunction in BD.

The major limitation of our study is the assessment of ED and the replacement of vitamin D in our patients in an open-label design. As vitamin D levels did not reach to therapeutic levels after replacement, other factors such as decreased disease activity might be associated with better endothelial assessments. However, the number of active vs inactive patients were similar in baseline compared to 3. month evaluation in our study population. FMD and CIMT measurements also did not correlate with disease activity, suggesting that inflammation had a limited role in our results.

In conclusion, we have shown for the first time that impaired endothelial function and CIMT is associated with vitamin D levels and can be improved in vitamin-D deficient BD patients with replacement therapy. As vitamin D deficiency seems to be observed in up to 60% of BD patients in Turkey, we suggest routine measurement of vitamin D in BD patients, with replacement therapy as required.

References

1. YAZICI H, FRESKO I, YURDAKUL S: Behçet's syndrome: disease manifestations, management, and advances in treatment. *Nat Clin Pract Rheumatol* 2007; 3: 148-55.
2. ARNSON Y, AMITAL H, SHOENFELD Y: Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis* 2007; 66: 1137-42.
3. CORRETTI MC, ANDERSON TJ, BENJAMIN EJ *et al*.; INTERNATIONAL BRACHIAL ARTERY REACTIVITY TASK FORCE. : Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39: 257-65.
4. GÜL A: Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2001; 19 (Suppl. 24): S6-12.
5. KIRAZ S, ERTENLI I, OZTÜRK MA, HAZNEDAROĞLU IC, CELIK I, CALGÜNERI M: Pathological haemostasis and "prothrombotic state" in Behçet's disease. *Thromb Res* 2002; 105: 125-33.
6. CHAMBERS JC, HASKARD DO, KOONER JS: Vascular endothelial function and oxidative stress mechanisms in patients with Behçet's syndrome. *J Am Coll Cardiol* 2001; 37: 517-20.
7. CANTORNA MT, MAHON BD: Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med* (Maywood) 2004; 229: 1136-42.
8. AUDRAN M, BRIOT K: Critical reappraisal of vitamin D deficiency. *Joint Bone Spine* 2010; 77: 115-9.
9. WELSH P, PETERS MJ, SATTAR N: Is vitamin D in rheumatoid arthritis a magic bullet or a mirage? The need to improve the evidence base prior to calls for supplementation. *Arthritis Rheum* 2011; 63: 1763-9.
10. VABULAS RM, AHMAD-NEJAD P, DA COSTA C *et al*.: Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 2001; 276: 31332-9.
11. TARCIN O, YAVUZ DG, OZBEN B *et al*.: Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab* 2009; 94: 4023-30.
12. DO JE, KWON SY, PARK S, LEE ES: Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behçet's disease. *Rheumatology* (Oxford) 2008; 47: 840-8.
13. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
14. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
15. CUTOLO M Vitamin D and autoimmune rheumatic diseases. *Rheumatology* (Oxford) 2009; 48: 210-2.
16. CUTOLO M, OTSA K, LAAS K *et al*.: Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. *Clin Exp Rheumatol* 2006; 24: 702-4.
17. ROSSINI M, MADDALI BONGI S, LA MONTAGNA G *et al*.: Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. *Arthritis Res Ther* 2010; 12: R216.
18. WELSH P, PETERS MJ, MCINNES IB *et al*.: Vitamin D deficiency is common in patients with RA and linked to disease activity, but circulating levels are unaffected by TNF-α blockade: results from a prospective cohort study. *Arthritis Rheum* 2011; 70: 1165-7.
19. HAMZAOUI K, BEN DHIFALLAH I, KARRAY E, SASSI FH, HAMZAOUI A: Vitamin D modulates peripheral immunity in patients with Behçet's disease. *Clin Exp Rheumatol* 2010; 28 (Suppl. 60): 50-7.
20. KANDI B, CICEK D, ILHAN N Vitamin levels in Behçet's disease. *J Dermatolog Treat* 2007; 18: 69-75.
21. HAMPTON KK, CHAMBERLAIN MA, MENON DK, DAVIES JA: Coagulation and fibrinolytic activity in Behçet's disease. *Thromb Haemost*.1991; 66: 292-4.
22. OFLAZ H, MERCANOGLU F, KARAMAN O *et al*.: Impaired endothelium-dependent flow-mediated dilation in Behçet's disease: more prominent endothelial dysfunction in patients with vascular involvement. *Int J Clin Pract* 2005; 59: 777-81.
23. PROTOGEROU AD, SFIKAKIS PP, STAMATELOPOULOS KS *et al*.: Interrelated modulation of endothelial function in Behçet's disease by clinical activity and corticosteroid treatment.*Arthritis Res Ther* 2007; 9: R90.
24. PELAJO CF, LOPEZ-BENITEZ JM, MILLER LC: Vitamin D and autoimmune rheumatologic disorders. *Autoimmun Rev* 2010; 9: 507-10.
25. CHOI B, LEE ES, SOHN S: Vitamin D3 ameliorates herpes simplex virus-induced Behçet's disease-like inflammation in a mouse model through down-regulation of Toll-like receptors. *Clin Exp Rheumatol* 2011; 29 (Suppl. 67): S13-9.
26. HONG SN, PARK JC, YOON NS *et al*.: Carotid artery intima-media thickness in Behçet's disease patients without significant cardiovascular involvement. *Korean J Intern Med* 2008; 23: 87-93.
27. OZTÜRK MA, UNVERDI S, OKTAR SO *et al*.: Vascular endothelial growth factor and carotid intima-media thickness in patients with Behçet's disease. *Clin Rheumatol* 2008; 27: 961-6.
28. HAMZAOUI K, MAÎTRE B, HAMZAOUI A: Elevated levels of MMP-9 and TIMP-1 in the cerebrospinal fluid of neuro-Behçet's disease. *Clin Exp Rheumatol* 2009; 27 (Suppl. 53): S52-7.
29. HAMZAOUI K, AYED K, HAMZA M, HAMZAOUI A: VEGF and mRNA VEGF expression in CSF from Behçet's disease with neurological involvement. *J Neuroimmunol* 2009; 213: 148-53.
30. PAY S, ABBASOV T, ERDEM H *et al*.: Serum MMP-2 and MMP-9 in patients with Behçet's disease: do their higher levels correlate to vasculo-Behçet's disease associated with aneurysm formation? *Clin Exp Rheumatol* 2007; 25 (Suppl. 45): S70-5.