The Effect of Vitamin D Supplementation on Inflammatory and Hemostatic Markers and Disease Activity in Patients with Systemic Lupus Erythematosus: A Randomized Placebo-controlled Trial

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ABSTRACT. ***Objective.*** Systemic lupus erythematosus (SLE) is a chronic multisystem inflammatory autoimmune disease. Vitamin D has potent immunomodulatory properties that support its use in the treatment of autoimmune conditions, including SLE. We assessed vitamin D status in patients with SLE and determined alterations in inflammatory and hemostatic markers and disease activity before and after vitamin D supplementation.

Methods. Patients with SLE (n = 267) were randomized 2:1 to receive either oral cholecalciferol 2000 IU/day or placebo for 12 months. Outcome measures included assessment of alterations in levels of proinflammatory cytokines and hemostatic markers, and improvement in disease activity before and after 12 months of supplementation. Disease activity was measured by the SLE Disease Activity Index. Vitamin D levels were measured by Liaison immunoassay (normal 30–100 ng/ml). Serum levels between 10 and 30 ng/ml were classified as vitamin D insufficiency and levels < 10 ng/ml as vitamin D deficiency.

Results. The mean 25(OH)D level at baseline was 19.8 ng/ml in patients compared to 28.7 ng/ml in controls. The overall prevalence of suboptimal and deficient 25(OH)D serum levels among patients with SLE at baseline was 69% and 39%, respectively. Lower 25(OH)D levels correlated significantly with higher SLE disease activity. At 12 months of therapy, there was a significant improvement in levels of inflammatory and hemostatic markers as well as disease activity in the treatment group compared to the placebo group.

Conclusion. Vitamin D supplementation in patients with SLE is recommended because increased vitamin D levels seem to ameliorate inflammatory and hemostatic markers and show a tendency toward subsequent clinical improvement. Clinical Trial Registry NCT01425775. (J Rheumatol First Release Dec 1 2012; doi:10.3899/jrheum.111594)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS — VITAMIN D
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It has become apparent that vitamin D has diverse biological effects beyond its established actions on bone and mineral homeostasis[1].

The discovery of vitamin D receptors on the surface of immune cells including antigen-presenting cells, natural killer cells, and B and T lymphocytes explains the multiple immunomodulatory effects on both innate and adaptive immune responses[2,3].

Vitamin D may play a key role in mediating communication between the innate and adaptive pathways of the immune system[4]. Crucially, this activity appears to be dependent on the availability of precursor vitamin D for localized activation, that is, the vitamin D status of an individual. Thus, both innate and adaptive immunity may be profoundly affected in patients who are vitamin D insufficient, leading to an increased risk for immune-mediated diseases[5,6].

A link has been established between vitamin D and the immune system and vitamin D has been studied as a modifiable environmental risk factor in autoimmune disease in animal models, including systemic lupus erythematosus (SLE). Significant epidemiological evidence has linked vitamin D status with autoimmune disease susceptibility and severity, although this has not yet been proven[7,8,9]. Low

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levels of vitamin D have been suspected as risk factors for the development of rheumatic diseases and the persistence of disease activity[4].

Vitamin D deficiency is highly prevalent worldwide and evidence is mounting that it contributes to the morbidity and mortality of multiple chronic diseases, including SLE[10]. A metaanalysis of 18 randomized controlled trials showed that participants randomized to vitamin D experienced fewer deaths compared to those randomized to placebo[11].

Vitamin D deficiency skews the immunological response toward loss of tolerance. Adding vitamin D *in vitro* reverses immunological abnormalities characteristic of SLE. Further, vitamin D supplementation has been shown to improve disease in murine models of SLE, rheumatoid arthritis, type 1 diabetes mellitus, and other autoimmune diseases[12]. In collagen-induced arthritis (CIA), vitamin D substitution reduced the severity of arthritis and, if given before immunization, prevented the development of CIA[13]. The exact physiological effect and clinical significance of vitamin D deficiency in SLE are still unclear, as are the specific effects of vitamin D and vitamin D supplementation on SLE.

"Prophylactic" treatment with this compound has been advocated by some authors to prevent perpetuation of autoimmune disease. Accordingly, the aim of our study was to assess vitamin D status in patients with SLE by measuring the levels of 25 hydroxyvitamin D [25(OH)D], and to determine alterations in inflammatory and hemostatic markers as well as disease activity before and after vitamin D supplementation.

MATERIALS AND METHODS

Study design. This was a randomized, double-blind, placebo-controlled study (Clinical Trial Registry NCT01425775) conducted at the University of Alexandria. The ethics committee of our institution approved it. All subjects gave informed consent and the procedures were in accord with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. Following informed consent, eligibility criteria and clinical status were assessed at the first visit.

Study participants. Initially, 325 individuals were screened. Two hundred sixty-seven patients with SLE who met eligibility criteria were enrolled (228 women, 39 men). Mean age was 38.8 years and mean disease duration was 8.2 years. All patients fulfilled at least 4 of the American College of Rheumatology (ACR) classification criteria for SLE[14,15]. One hundred seventy-five healthy volunteers matched by age, sex, and body mass index recruited from hospital staff and visitors served as controls. The purpose of the control group was to provide normal reference baseline values for inflammatory, hemostatic, and immunologic measures and to ascertain the differences in vitamin D status.

Inclusion criteria. Inclusion criteria included premenopausal women and men fulfilling 4 ACR criteria for SLE, attending the outpatient clinic of our institution, and having SLE Disease Activity Index (SLEDAI) ≥ 1 and a baseline concentration of 25(OH)D < 30 ng/ml.

Exclusion criteria. Subjects were excluded from participation if they had other autoimmune, chronic inflammatory, or infectious conditions; a history of renal stones, liver disease, or hypercalcemia; serum creatinine > 2.5 g/dl; or if they were pregnant.

Randomization and blinding. All subjects and physicians were blinded to group assignment and treatment allocation. Patients were randomized into 2 parallel groups, using a ratio of 2:1 of vitamin D supplementation to placebo, for 12 months of treatment. This method of randomization was selected to optimize therapy and to increase the number of patients receiving therapy.

Assessment and treatment protocol. All subjects gave a detailed history and had a thorough physical examination. Clinical data and lifestyle characteristics were obtained by questioning and from patients' charts, including disease duration, body mass index (BMI), and medication usage including mean daily dosage of corticosteroids (CS). Diet content was assessed by a food frequency questionnaire for daily intake of calcium. Specific questions about sun-avoidance practices of patients, including current use of sun protection, mean daily duration of sun exposure, and presence of photosensitivity, were asked to account for skin production of vitamin D. Following a thorough baseline clinical evaluation, patients were randomized into 2 groups. The first group consisted of 178 patients, assigned to receive 2000 IU of cholecalciferol in the form of tablets (oral vitamin D_3) containing fixed combinations of calcium and cholecalciferol for 12 months, and the second group consisted of 89 patients who were given identical placebo tablets for 12 months. The rationale for the dosage of vitamin D stems from data reported for healthy adults. One report recommends that a supplement of 2000 IU of vitamin D_3/day would substantially increase the prevalence of optimal 25(OH)D levels and lead to better health outcomes[16]. Given the suboptimal status reported in patients with SLE, it seemed appropriate to extend these recommendations to patients with SLE.

Both groups were allowed to continue their ongoing standard therapy [CS < 10 mg/day, hydroxychloroquine (HCQ), immunosuppressants, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers], provided the dosages were kept constant. None of the patients were taking a statin at the time of the study and none were taking vitamin D prior to entry into the study. At the time of vitamin D assay, disease activity was measured by the SLEDAI 2000 (SLEDAI-2K), a validated measure of disease activity[17].

Interventions. Blood sampling was done from April to September 2011, to provide the highest vitamin D levels. All blood samples were collected at 8 A.M. Patients were matched for age, sex, BMI, ethnicity, calcium intake, duration of disease, and mean daily dose of CS.

Disease activity (SLEDAI) was measured on the day the serum samples were drawn. Levels of vitamin D and 25(OH)D_3 were analyzed in serum samples, in duplicate, using the Liaison 25OH immunoassay (Diasorin). This is a competitive 2-step chemiluminescence assay having a measuring range of 4–150 ng/ml. The reported specificity for 25(OH)D_3 is 100%. Normal levels of vitamin D range from 30 to 100 ng/ml. Serum levels between 10 and 30 ng/ml were classified as vitamin D insufficiency, and levels < 10 ng/ml as vitamin D deficiency. Levels > 30 ng/ml are recommended to avoid parathyroid hormone activation. We set 10–30 ng/ml as the level for insufficiency based on previous studies and the 2008 guidelines[15,16].

SLE-related markers measured included levels of anti-dsDNA and antinuclear antibodies (ANA), anti-Sm, anticardiolipin antibodies, and complement 4 (C4). Erythrocyte sedimentation rate (ESR) was also measured. ANA were measured using a human epithelial cell line 2 indirect immunofluorescent assay (Inova Diagnostics) according to the manufacturer's instructions. Anti-dsDNA antibodies were detected using an indirect immunofluorescent assay (Inova Diagnostics) according to the manufacturer's instructions. Serum concentrations of autoantibodies were analyzed by ELISA according to the manufacturer's instructions. ESR was measured using the Westergren method; von Willebrand factor (vWF; Stago) and fibrinogen (Fibri-test) were measured using commercial kits. Concentrations of the proinflammatory cytokines interleukin 1 (IL-1), IL-6, IL-18, and tumor necrosis factor (TNF-α) were measured in serum samples with commercial sandwich ELISA kits (Quantikine, R&D Systems) following the manufacturer's instructions. The concentrations of samples were determined using a standard curve obtained from the optical densities of

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standards with known concentration. C4 levels were measured according to the manufacturer's instructions (Dialab GmbH) and analyzed using a BN II nephelometer. Serial measurements of these markers were made at 3-month intervals.

Safety and tolerability were also assessed at every visit. Treatment-related adverse events reported by patients were collected at each visit. A treatment-related adverse event was any reported event first occurring or worsening in severity during treatment, compared to the baseline period.

Study endpoints and outcome measures. Primary outcome measures included the assessment of alterations in levels of the proinflammatory cytokines and hemostatic markers before and after 12 months of vitamin D supplementation. Secondary outcome measure was the improvement in disease activity (SLEDAI) following 12 months of therapy.

Statistical analyses. Data were analyzed using the SPSS software, version 16.0. All analyses were conducted on an intent-to-treat basis. The trial was designed to randomize 300 individuals to achieve 250 evaluable individuals at the end of 6 months. A total sample size of 250 was predicted to provide a power of 90% to detect a 25% difference in the outcome measures between the intervention and placebo groups. Values in this study are expressed as mean (SD) or numbers (percentages). Continuous variables between the 2 groups were compared using the Student's t-test. Categorical groups were compared by the chi-squared test and Fisher's exact test, as appropriate. Spearman's rank correlation coefficient was used to find the relationship between 25(OH)D levels and disease activity (SLEDAI) scores. Multivariate regression was used to adjust for potential codeterminants of vitamin D including season, use of HCQ and immunosuppressants (AZA), serum creatinine, SLEDAI, anti-dsDNA, C4, and inflammatory and hemostatic markers, with 25(OH)D as the dependent variable. Multivariate linear regression was used when 25(OH)D was a continuous variable and multivariate logistic regression was used when 25(OH)D was a categorical variable. Statistical significance was defined as a 2-sided p value < 0.05.

RESULTS

The study procedure is shown in Figure 1. The 267 eligible participants were randomized into an intervention group (n = 178) and a placebo group (n = 89) and followed for 12 months. Of the 267 patients, 236 (88%) completed the study. Demographic characteristics of the study group are depicted in Table 1. The baseline characteristics were similarly distributed between the 2 treatment groups. The mean levels of proinflammatory, hemostatic, and disease activity markers before and after vitamin D supplementation are shown in

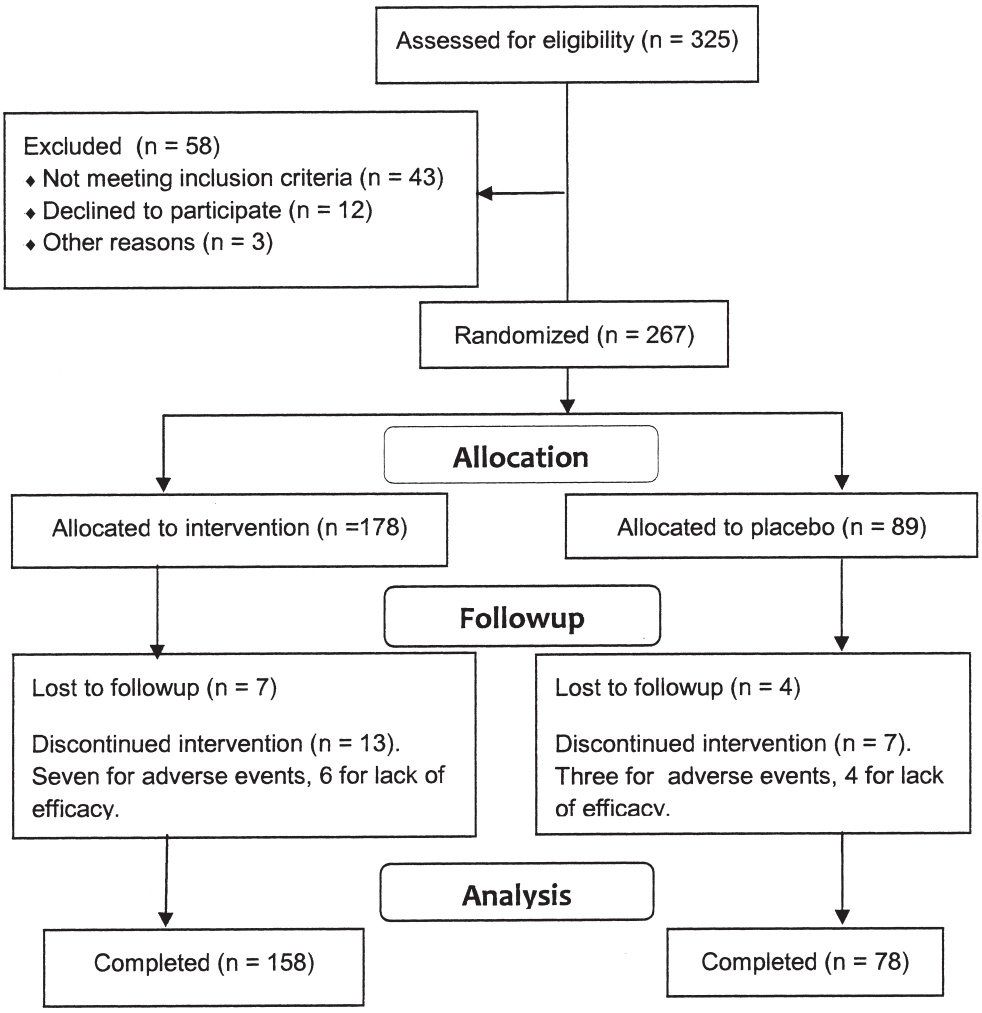

Figure 1. The procedure of the study.

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Table 1. Baseline characteristics of study participants according to treatment group. Values are number (%) unless otherwise indicated.

Characteristic	Intervention, n = 178	Placebo, n = 89	p
Age, yrs, mean (SD)	38.8 (5.7)	38.9 (3.5)	0.78
Sex, female/male, no.	152/26	76/13	—
Body mass index, kg/m^2, mean (SD)	24.1 (6.6)	23.9 (7.1)	0.19
Dietary calcium, mg/day, mean (SD)	637 (329)	635 (338)	0.64
25 hydroxyvitamin D, ng/ml, mean (SD)	19.9 (16.3)	19.8 (16.7)	0.71
Serum creatinine, mg/dl, mean (SD)	1.3 (0.4)	1.1 (0.7)	0.85
Serum calcium, mg/dl, mean (SD)	9.3 (0.5)	9.3 (0.4)	0.22
Disease duration, yrs, mean (SD)	8.3 (6.9)	8.2 (6.7)	0.17
Current sun protection use	121 (68)	61 (69)	0.56
Sun exposure/day,			
≥ 15 min	138 (78)	70 (79)	0.58
≤ 15 min	40 (22)	19 (21)	0.45
Photosensitivity	118 (66)	59 (66)	0.59
SLEDAI score at vitamin D measurement, mean (SD)	4.8 (3.4)	4.7 (3.6)	0.39
ANA-positive (%)	81	80	0.65
Anti-dsDNA-positive (%)	86	85	0.87
Anti-Sm positive (%)	25	24	0.66
Anti-cardiolipin IgG-positive (%)	22	21	0.65
IgM-positive (%)	18	17	0.59
C4, mg/l, mean (SD)	0.166 (0.091)	0.168 (0.089)	0.65
Medications			
Corticosteroids, mg, mean dose (SD)	10.0 (6.1)	9.9 (5.8)	0.48
Antimalarial use (HCQ)	144 (81)	72 (81)	0.51
Immunosuppressants (AZA)	46 (26)	24 (27)	0.46
ACE inhibitors/ARB	48 (27)	25 (28)	0.55
Renal disease	45 (25)	22 (25)	0.33
Nervous system	14 (8)	6 (7)	0.71
Serositis	16 (9)	9 (10)	0.82
Hematological	117 (66)	58 (65)	0.47
Polyarthritis	115 (65)	59 (66)	0.39
Skin involvement	110 (62)	55 (62)	0.75

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ANA: antinuclear antibodies; AZA: azathioprine; HCQ: hydroxychloroquine; ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blockers.

Table 2. At baseline all markers were significantly higher in patients with SLE compared to healthy controls. At 12 months of therapy, there was a significant decrease in levels of inflammatory, hemostatic, and disease activity markers in the vitamin D group compared to the placebo group. The mean vitamin D levels of the study population at baseline and after 12 months of intervention are shown in Table 3. The mean 25(OH)D level at baseline was 19.8 ng/ml in patients compared to 28.7 ng/ml in controls. The overall prevalence of suboptimal 25(OH)D levels at baseline among patients with SLE was 69% compared to 33% after 12 months of vitamin D supplementation. Insufficient 25(OH)D serum levels were found in 69% of patients in the intervention group compared to 19% after 12 months of therapy. Deficient 25(OH)D serum levels were found in 33% of patients with SLE in the intervention group at baseline, and after 12 months of therapy none of the patients in the intervention group had deficiency (Table 3). Within-group analysis of the data of active disease by SLEDAI score reduction showed that 46/122 patients (38%) with vitamin D insufficiency and 23/58 patients (40%) with vitamin D deficiency in the intervention group had a reduction in SLEDAI ≥ 3. One-third of the patients with SLE did not reach optimal vitamin D levels. Within-group analysis demonstrated that these patients had renal disease, were taking a higher dosage of HCQ [conversion of 25(OH)D into 1,25$(OH)_2$D is reduced by drugs such as HCQ and by renal disease], and had darker skin tone [melanin is known to inversely correlate with endogenous production of vitamin D and lower serum levels of 25(OH)D]. Inflammatory/serologic biomarkers were significantly higher than in those patients who had achieved optimal levels of vitamin D.

Levels of 25(OH)D correlated inversely with SLE disease activity scores ($r = –0.583$, $p < 0.05$). Table 4 depicts significant improvement in disease activity (SLEDAI) in the vitamin D group (both insufficiency and deficiency) versus the placebo group at 12 months.

The changes occurring in the SLE-related antibodies before and after 12 months of treatment are shown in Table

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Table 2. Mean levels of proinflammatory cytokines, and hemostatic and disease activity markers of the study participants at baseline and after 12 months. Values are mean (SD).

Measure	Intervention, n = 178	Placebo, n = 89	Controls, n = 175
IL-1, pg/ml			
Baseline	0.59 (0.91)*	0.60 (0.92)*	0.35 (0.45)
12 mo	0.43 (0.53)**	0.61 (0.89)	
IL-6, pg/ml			
Baseline	8.85 (6.75)*	8.82 (6.71)*	1.18 (2.21)
12 mo	5.11 (7.11)**	7.96 (7.77)	
IL-18, pg/ml			
Baseline	485.22 (199.45)*	483.01 (199.91)*	224.61 (89.93)
12 mo	400.05 (192.37)**	482.87 (198.34)	
TNF-α, pg/ml			
Baseline	8.80 (8.22)*	8.43 (8.12)*	0.94 (1.13)
12 mo	4.63 (7.79)**	8.01 (7.58)	
ESR, mm/h			
Baseline	35.52 (9.92)*	32.25 (10.11)*	10.6 (1.1)
12 mo	15.11 (4.47)**	30.18 (9.99)	
Anti-dsDNA, U/ml			
Baseline	55.8 (14.1)*	55.6 (14.2)*	11.8 (12.2)
12 mo	32.8 (12.7)**	44.8 (13.9)	
Anti-Sm, U/ml			
Baseline	10.79 (9.01)*	10.75 (8.81)*	0.9 (1.1)
12 mo	8.21 (7.82)**	9.99 (7.94)	
C4, mg/l			
Baseline	0.166 (0.091)	0.168 (0.089)	NA
12 mo	0.270 (0.071)**	0.171 (0.079)	
Fibrinogen, mg/dl			
Baseline	335.17 (99.01)*	332.42 (105.14)*	186.21 (48.11)
12 mo	259.97 (88.65)**	334.89 (98.93)	
vWF, pg/ml			
At baseline	228.23 (128.16)*	229.10 (127.81)*	132.58 (68.59)
12 mo	200.61 (106.35)**	233.44 (117.65)	

* Significantly different from control group. ** Significantly different from placebo group. NA: not applicable; vWF: von Willebrand factor; ESR: erythrocyte sedimentation rate; TNF-α: tumor necrosis factor-α.

Table 3. Serum vitamin D levels at baseline and at 12 months. Values are number (%) unless otherwise indicated.

Serum Vitamin D	All Patients with SLE, n = 267	Intervention, n = 178	Placebo, n = 89
Baseline			
Mean 25(OH)D, ng/ml, mean (SD)	19.8 (16.5)*	19.9 (16.3)	19.7 (16.7)
25(OH)D < 30 ng/ml	183 (69)	122 (69)	61 (68)
25(OH)D < 10 ng/ml	87 (33)	58 (33)	29 (33)
At 12 months			
Mean 25(OH)D, ng/ml	33.9 (16.7)*	37.8 (16.3)**	19.9 (16.2)
25(OH)D < 30 ng/ml	88 (33)	34 (19)	54 (61)
25(OH)D < 10 ng/ml	30 (11)	0 (0)	30 (34)

* Significantly different from control group. ** Significantly different from placebo group.

Table 4. Variations in SLEDAI score according to 25(OH)D levels in patients on vitamin D supplementation versus patients taking placebo. Values are mean (SD).

Treatment Group	SLEDAI at Baseline	SLEDAI at 12 Months	p
Vitamin D supplementation			
Insufficiency	4.9 (3.6)	3.2 (2.8)	0.01
Deficiency	4.9 (3.5)	3.0 (2.5)	0.05
Placebo			
Insufficiency	4.8 (3.5)	4.5 (3.9)	0.69
Deficiency	4.9 (3.1)	4.6 (3.3)	0.47

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

5. Anti-Sm and anti-dsDNA levels decreased significantly in the vitamin D group compared to the placebo group at 12 months. An increase in C4 levels was observed in the vitamin D group compared to the placebo group at 12 months. In adjusted linear regression models, lower serum 25(OH)D concentrations were associated with higher average SLEDAI scores ($r = –0.265$; $p = 0.01$ for trend). Lower serum 25(OH)D concentrations were also associated with increased levels of anti-dsDNA ($r = –0.333$; $p < 0.01$), anti-Sm ($r = –0.475$; $p < 0.05$), IL-1 ($r = –0.322$; $p < 0.01$), IL-6 ($r = –0.264$; $p < 0.05$), IL-18 ($r = –0.358$; $p < 0.05$), TNF-α ($r = –0.413$; $p < 0.01$), ESR ($r = –0.492$; $p < 0.05$), fibrinogen ($r = –0.472$; $p < 0.05$), and vWF ($r = –0.521$; $p < 0.05$). Lower serum 25(OH)D concentrations were

Table 5. Alterations in the SLE-related antibodies before and after 12 months of treatment. Values are mean (SD) unless otherwise indicated.

SLE-related Antibodies	Vitamin D Group	Placebo Group	p
Anti-dsDNA, U/ml			
Baseline	55.8 (14.1)	55.6 (14.2)	0.67
% positive	86	85	
12 months	32.8 (12.7)	44.8 (13.9)	0.05
% positive	67	82	
Anti-Sm, U/ml			
Baseline	10.79 (9.01)	10.75 (8.81)	0.66
% positive	25	24	
12 months	8.21 (7.82)	9.59 (7.94)	0.05
% positive	16	22	
C4 (mg/l)			
Baseline	0.166 (0.091)	0.168 (0.089)	0.65
12 months	0.270 (0.071)	0.179 (0.079)	0.05
Anticardiolipin IgG, GPL IU/ml			
Baseline	11.1 (2.3)	11.3 (2.5)	0.66
% positive	22	21	
12 months	10.9 (2.1)	11.1 (2.0)	0.59
% positive	18	19	
Anticardiolipin IgM, GPL IU/ml			
Baseline	7.9 (2.4)	7.8 (2.2)	0.61
% positive	18	17	
12 months	7.5 (2.1)	7.6 (2.5)	0.65
% positive	14	16	

ANA: antinuclear antibodies.

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associated with lower C4 levels ($r = 0.458$; $p < 0.05$). Data showed significantly higher disease activity among patients with severe vitamin D deficiency (25(OH)D < 10 ng/ml) compared with less deficient vitamin D levels. Increased disease activity expressed by SLEDAI scores was associated with increased serum IL-1, IL-6, TNF-α, and low 25(OH)D levels in patients with SLE. None of the patients had to increase their immunomodulatory treatments during the study period. Mild to moderate flares were recorded in 24% of the placebo group compared to 10% of the intervention group ($p < 0.005$).

The drug was well tolerated with no serious adverse events recorded. The main treatment-related adverse events recorded were constipation (4% in intervention vs 2% in placebo group); anorexia (2% in intervention vs 1% in placebo group), hypercalcemia (2% in intervention vs 0% in placebo group), and hypercalciuria (2% in intervention vs 0% in placebo group). Six patients in the intervention group and 4 in the placebo group dropped out on followup because of lack of efficacy.

DISCUSSION

Increasing evidence suggests a pivotal role for vitamin D in the pathogenesis and progression of autoimmune diseases such as SLE. In our study, we found a high prevalence of vitamin D insufficiency and vitamin D deficiency among patients with SLE. This high prevalence of suboptimal 25(OH)D levels has been reported in several studies[18,19,20,21]. Following vitamin D supplementation, 25(OH)D levels increased significantly in the patients with SLE who previously had insufficient and deficient levels.

In addition, a significant inverse correlation between 25(OH)D levels and disease activity markers was seen. The 25(OH)D levels were lowest among patients with active SLE. We have thus demonstrated that vitamin D deficiency may result in increased activity in SLE. It is plausible to suggest that patients with SLE who have more active disease are prone to vitamin D deficiency as a result of avoidance of sunshine, use of photoprotection, renal insufficiency, and chronic use of HCQ and glucocorticoids, drugs known to alter vitamin D metabolism.

It is possible that the low vitamin D levels observed are indicative of an ongoing inflammatory process. Inflammation *per se* potentially enhances vitamin D metabolism. Several studies report a similar inverse relationship between 25(OH)D levels and disease activity[22,23], yet other studies failed to show a similar correlation[24,25]. This may be explained by several factors including sample size, patient characteristics, and seasonal variation.

Following 12 months of vitamin D supplementation in the intervention group compared to the placebo group, there was a significant improvement in disease activity scores as well as a significant reduction in the levels of autoantibodies (anti-Sm, anti-dsDNA) and ESR levels, with a rise in the levels of C4. Patients in the intervention group had mild/low disease activity and showed a trend toward improvement in their SLEDAI score. Further, patients with significantly higher disease activity had more severe vitamin deficiency. This suggests that patients with higher SLEDAI would benefit from vitamin D supplementation even more than those with lower SLEDAI scores. As to the manifestations that would potentially benefit the most, our study supports the hypothesis that vitamin D supplementation may help normalize immunological abnormalities of patients with SLE, and offers insight into an "immuno-inflammatory-modulatory" mechanism (with improvement in immune-inflammatory and hemostatic markers) for vitamin D. Consequently, improving vitamin D status among patients with SLE could benefit most of the common manifestations.

The exact mechanism by which vitamin D affects the pathogenesis of SLE remains unclear. Vitamin D supplementation may have a role in the maintenance of B cell homeostasis[26], and correction of vitamin D levels may thus be useful in the treatment of B cell-mediated conditions such as SLE. Vitamin D deficiency seems to contribute to increased B cell activation in patients with SLE and increased production of autoantibodies in genetically susceptible individuals.

Inflammation enhances vitamin D catabolism, and increasing vitamin D levels seems to decrease activity[26]. Low vitamin D levels are consistent with an ongoing immunoinflammatory process. Increasing serum levels of 25(OH)D appear to provide an antiinflammatory effect and improve clinical disease activity. Several studies suggest that administration of vitamin D ameliorates inflammation in animal models of autoimmune diseases including SLE[11]. The animal studies are complemented by human epidemiologic studies demonstrating an inverse correlation between vitamin D intake with risk of several autoimmune diseases[9,13].

Further, we observed a significant improvement in the levels of proinflammatory cytokines following 12 months of vitamin D supplementation compared to placebo. Multiple factors appear be involved in the pathophysiology of SLE including immunoinflammatory processes. Changes in the concentration of proinflammatory mediators such as IL-1, IL-6, IL-18, and TNF-α have been described in patients with SLE. SLE is a multisystemic chronic inflammatory disease. Many of the immunological observations such as defective T cell regulation of B cell activity, overproduction of autoantibodies, and reduced phagocytic clearance of immune complexes and apoptotic bodies are the opposites of the actions of calcitriol[27,28].

Vitamin D has a key role in preserving immune homeostasis. Vitamin D promotes immune stability in the innate and adaptive immune systems, preventing lapses toward autoimmunity. Immunological effects of vitamin D include

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decreasing the Th1 CD4+ T cells and cytokines, increasing regulatory T cells, downregulating T cell-driven IgG production, inhibiting the differentiation of dendritic cells, and preventing the proliferation of activated B cells[29].

The overall effect of vitamin D is enhancement of protective innate immune response, while maintaining self-tolerance by dampening overactive adaptive immune responses[30]. Amelioration of proinflammatory cytokines by vitamin D supplementation may be attributed to the anti-inflammatory and immunomodulation effect of vitamin D. All measured inflammatory and serologic markers (except for anticardiolipin IgG/IgM levels, which did not decrease to significant levels) decreased after vitamin D supplementation. The proposed mechanism for these cytokines to decrease with vitamin D supplementation is that the biologically active calcitriol mediates its effects through the vitamin D receptors (VDR) on immune cells, suggesting that vitamin D has modulatory effects on both the innate and adaptive immunity. The discovery of VDR on immune cells suggests many immunomodulatory effects of vitamin D. Vitamin D enhances chemotaxis and phagocytosis of macrophages and promotes the development of regulatory T cells (Treg) through an increase in cytokines that leads to a shifting of the polarization of T cells from Th1 and Th17 toward a Th2 phenotype, as well as by inhibiting the differentiation of B cells into plasma cells[29]. Hormonally active vitamin D inhibits several components of the immune system including dendritic cell differentiation and maturation, B cell differentiation, T cell proliferation in response to T cell receptor stimulation, and secretion of TNF-α[29,30].

Vitamin D may thus be regarded as a potential immunosuppressive antiinflammatory agent. Ritterhouse, *et al* observed that vitamin D deficiency is associated with immune abnormalities in SLE, suggesting that vitamin D plays a role in autoantibody production in SLE[31].

Further, we observed an increase in vWF and fibrinogen levels in patients with SLE compared to controls at baseline. Levels decreased significantly in the intervention group following vitamin D supplementation compared to placebo. These findings suggest that vitamin D may have a role in maintaining antithrombotic homeostasis and in determining a thrombolytic profile before progression to cardiovascular disease, the leading cause of death in patients with SLE.

Hypovitaminosis D appears to contribute to a chronic inflammatory, thrombolytic state and may have a role among other factors in increasing the presence of SLE. We have demonstrated that vitamin D supplementation at a dose of 2000 IU daily for 12 months increases 25(OH)D levels to a substantial degree and into a sufficient range. The limitations of our study include insufficient data as to the optimal dosage of vitamin D, particularly because a substantial number of patients with SLE in the intervention group still displayed suboptimal levels despite supplementation with vitamin D. Also, we did not include a flare tool in our study.

Our findings demonstrated that in patients receiving vitamin D compared to placebo, in addition to standard background SLE therapy, higher levels of 25(OH)D improved disease activity and amelioration of proinflammatory and hemostatic markers. Vitamin D, a safe, inexpensive, and widely available agent, may be effective as a disease-suppressing intervention for patients with SLE. In addition to the potential benefit of vitamin D replacement on improvement of SLE activity, vitamin D seems to have an immune-inflammatory-modulatory role that may benefit musculoskeletal and cardiovascular manifestations of SLE. This role could also help maintain immune health, thus avoiding the excess morbidity and mortality associated with vitamin D deficiency. We recommend routine assessment of vitamin D levels and adequate supplementation of the vitamin in patients with SLE. However, further large-scale studies are needed to establish the desired level of immune homeostasis, through periodic longitudinal assessments of vitamin D status with dose-response properties of vitamin D supplementation.

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