# A study on the role of calcium homeostasis and vitamin D deficiency in premenopausal systemic lupus erythematosus patients and its relation with disease activity

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**Introduction**
Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder that may affect multiple organ systems. Vitamin D levels and its role in lupus inflammation is still a matter of debate.
**Objective**
The aim of this study was to assess the role of calcium homeostasis and vitamin D deficiency in premenopausal SLE patients and its relation with disease activity.
**Patients and methods**
We assessed serum 25-hydroxyvitamin D [25(OH)D] level in 60 (SLE) patients and 20 age and sex-matched healthy controls. We also assessed different clinical, immunological, and laboratory disease parameters in SLE patients – namely, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibody, antidouble stranded DNA, C3, and C4–and disease activity score using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. We correlated serum 25(OH)D with disease activity and different environmental parameters that might affect 25(OH)D level.
**Results**
A significantly lower 25(OH)D level was found in SLE patients compared with controls (P=0.033). Serum 25(OH)D was inversely correlated to SLEDAI score (P=0.043), antidouble stranded DNA (P<0.001), and erythrocyte sedimentation rate (P<0.001), but directly correlated to C3 and C4 levels (P=0.029). There was an inverse correlation between vitamin D supplementation and SLEDAI score (MCP=0.030), but there was no significant correlation with both calcium supplementation (P=0.861) and ionized calcium (P=0.681).
**Conclusion**
Vitamin D insufficiency and deficiency is highly prevalent in SLE patients than in healthy controls, and is prevalent among SLE patients with higher disease activity, which suggests an important role of vitamin D3 in the pathogenesis of SLE disease activity and flares. The therapeutic effect of vitamin D in SLE should be further assessed in interventional studies.

**Keywords:**
premenopausal women, systemic lupus erythematosus, vitamin d

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## Introduction

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder that may affect multiple organ systems [1,2]. It is characterized by a myriad of immune system aberrations that involve B cells, T cells, and cells of the monocytic lineage, resulting in polyclonal B cell activation, increased numbers of antibody-producing cells, hypergammaglobulinemia, autoantibody production, and immune complex formation [3]. Multiple factors are associated with the development of the disease, including genetic, racial, hormonal, and environmental factors. Sun exposure as one of the environmental risk factors plays an important role in the pathogenesis of SLE [4]. As many as 70% of SLE patients have disease flared on exposure to ultraviolet light [5].

Vitamin D is a fat-soluble vitamin that is synthesized in human skin exposed to ultraviolet radiation. Besides its classical effects on bone and calcium homeostasis, vitamin D has progressively become recognized as a pluripotent regulator of many other biological functions [6]. This is supported by vitamin D

receptor being widely, although not universally, distributed throughout different tissues of the body.

Several reports pointed out a putative role for the active metabolite 1,25-hydroxyvitamin $D_3$ [1,25(OH)$D_3$] in immune system regulation, exerting concentration-dependent anti-inflammatory autocrine and paracrine effects in lymphoid microenvironments [7], with an inhibitory effect on dendritic cells, CD4, CD8, B lymphocytes, and the production of cytokines such as interferon (IFN), interleukin (IL)-2, IL-6, and tumor necrosis factor (TNF). Moreover, 1,25(OH)$D_3$ increases the number of T regulatory cells and synthesis of other cytokines such as IL-4, IL-10, and transforming growth factor (TGF) [8]. Evidence accumulated in recent years suggests an important role for vitamin D in the regulation of immune response and as a modifiable environmental factor in autoimmune diseases [7].

The association between vitamin D and SLE was first described in 1979 [9]. Several studies worldwide have reported that vitamin D deficiency is more prevalent among SLE patients compared with the general population [10]. This can be attributed to the fact that patients with SLE are advised to avoid direct sunlight, a common trigger of disease flares and the primary source of vitamin $D_3$ [11]. In addition, other lupus-related factors that may contribute to vitamin D deficiency include renal disease [12] and the use of steroids that are thought to alter the metabolism of vitamin D [13]. Recent studies also showed that SLE patients produce antivitamin D antibodies [14].

## Aim of the work

The aim of this study was to investigate the role of calcium hemostasis and vitamin D deficiency among premenopausal SLE patients and to determine its relation with disease activity.

## Patients and methods

This study was conducted on sixty patients diagnosed according to the Systemic Lupus International Collaborating Clinics Classification criteria for the diagnosis of SLE [15]. In addition, 20 age and sex-matched healthy individuals were included. Patients were recruited from the outpatient clinic or the inpatient ward of the Internal Medicine Department at Alexandria University Hospitals. Other conditions such as chronic liver disease, gastrointestinal surgery, smoking, metabolic bone disease, malabsorption syndrome, and autoimmune diseases other than lupus were excluded.

All patients were subjected to detailed history taking, including drug history (calcium supplementation, steroids, and vitamin D supplementation). A questionnaire was applied to all patients comprising details on the following: sun exposure, including outdoor activities and dress style; and food habits, including type (vegetables, fish, and dairy products) and number of servings per week. All patients were subjected to complete physical and muscoskeletal examination. In addition, clinical assessment of disease activity with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was [2] applied for all patients. Laboratory evaluation included the following: complete blood picture, liver enzymes (alanine transaminase and aspartate aminotransferase), serum albumin, renal function test (blood urea and serum creatinine), complete urine analysis, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), C3, C4, antinuclear antibodies titer, antidouble stranded DNA antibodies (anti-ds-DNA) titer, serum calcium (total-ionized), serum parathyroid hormone level (PTH), and serum 25(OH)D.

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsiniki and informed consent was obtained from each patient.

### Statistical analysis

Data were checked, entered, and analyzed using the SPSS 18 software package (SPSS Inc., Chicago, Illinois, USA). The normally distributed data were expressed as mean± SD. Multiple group comparisons were performed using one-way analysis of variance. Univariate correlations between study variables were calculated with Spearman's rank correlation coefficients ($r$). $P$-values less than 0.05 were considered significant.

## Results

In the present study, group I included 60 premenopausal SLE patients, with a mean age of 28.75±6.62 years. The mean duration of disease was 36.57±46.29 months. Group II included 20 healthy individuals, with a mean age of 27.35±5.10 years. There was no significant difference as regards age and sex between the two groups ($P$=0.391) (Tables 1 to 3).

The mean value for C3 was 76.11±37.75 mg/dl, with normal values in 26 patients (43.3%), and low values in 34 patients (56.7%). The mean value for C4 was 19.08± 14.67 mg/dl, with normal values in 37 patients (61.7%), and low values in 23 patients (38.3%). The mean value for antinuclear antibodies was 286.33±182.62 IU; all 60

**Table 1 Comparison between the two groups according to demographic data (n=80)**

| Variables | Group I patients (*n*=60) | Group II control (*n*=20) | *t* | *P* |
|---|---|---|---|---|
| Age (years) | | | | |
| Minimum–maximum | 14.0–40.0 | 18.0–37.0 | 0.862 | 0.391 |
| Mean±SD | 28.75±6.62 | 27.35±5.10 | | |
| Median | 30.0 | 28.50 | | |
| Duration disease (months) | | | | |
| Minimum–maximum | 0.25–192.0 | – | | |
| Mean±SD | 36.57±46.29 | – | – | – |
| Median | 12.0 | – | | |

**Table 2 Distribution of the studied cases according to symptoms (*n*=60)**

| Symptoms | *n* (%) |
|---|---|
| Oral ulcers | 35 (58.3) |
| Photosensitivity | 34 (56.7) |
| Hair fall | 34 (56.7) |
| Malar rash | 27 (45.0) |
| Fever and malaise | 26 (43.3) |
| Arthritis and arthralgia | 18 (30.0) |
| Respiratory | 10 (16.7) |
| Hypertension | 10 (16.7) |
| Cardiovascular | 9 (15.0) |
| LL edema | 6 (10.0) |
| Discoid rash | 2 (3.3) |
| Seizures | 0 (0.0) |
| Psychosis | 0 (0.0) |

LL, lower limb.

patients had positive results. The mean value for anti-ds-DNA was 159.88±93.99 IU/l, with positive results in 58 patients (96.7%) and negative results in two patients (3.3%), in which the seroconversion was secondary to treatment (Table 4).

In group I, 26 patients (43.3%) had no disease activity, 17 patients (28.3%) had mild disease activity, five patients (8.3%) had moderate disease activity, and 12 patients (20.0%) had severe disease activity. The mean value for SLEDAI score was 6.63±5.74. Disease severity was classified based on SLEDAI score as follows: no disease activity, SLEDAI score 0–3; mild disease activity, score 4–8, moderate disease activity, score 8–12, and severe disease activity, score greater than12 (Table 5 and Fig. 1).

In group I, the mean value of 25(OH)D was 12.08 ±9.41 ng/ml; 35 patients (58.3%) had deficient levels of 25(OH)D, 18 patients (30.0%) had insufficient levels, and seven patients (11.7%) had sufficient levels. However, in group II, the mean value of 25(OH)D was 15.90±9.44 ng/ml: eight patients (40.0%) had deficient levels, eight patients (40.0%) had insufficient

**Table 3 Distribution of the studied cases according to their laboratory data (*n*=60)**

| Variables | *n* (%) |
|---|---|
| Hemoglobin (11–16) (g/dl) | |
| Normal | 12 (20.0) |
| Abnormal | 48 (80.0) |
| Minimum–maximum | 6.60–13.40 |
| Mean±SD | 9.67±1.63 |
| Median | 9.70 |
| WBC (4–11) (×$10^3$ cells/$mm^3$) | |
| Normal | 35 (58.3) |
| Abnormal | 25 (41.7) |
| Minimum–maximum | 1.50–19.30 |
| Mean±SD | 6.02±3.75 |
| Median | 4.95 |
| PLT (150–450) (×$10^3$ cells/$mm^3$) | |
| Normal | 38 (63.3) |
| Abnormal | 22 (36.7) |
| Minimum–maximum | 39.0–3000.0 |
| Mean±SD | 263.8 ±378.4 |
| Median | 221.0 |
| SGOT (AST) (15–37) (μ/l) | |
| Minimum–maximum | 15.0–37.0 |
| Mean±SD | 23.80±6.53 |
| Median | 24.0 |
| SGPT (ALT) (30–65) (μ/l) | |
| Minimum–maximum | 30.0–61.0 |
| Mean±SD | 39.77±7.10 |
| Median | 38.50 |
| Total protein (6.4–8.0) (g/dl) | |
| Normal | 57 (95.0) |
| Abnormal | 3 (5.0) |
| Minimum–maximum | 6.0–8.0 |
| Mean±SD | 7.16±0.48 |
| Median | 7.10 |
| Serum albumin (3.4–5.0) (g/dl) | |
| Normal | 55 (91.7) |
| Abnormal | 5 (8.3) |
| Minimum–maximum | 1.90–5.0 |
| Mean±SD | 3.91±0.59 |
| Median | 3.90 |
| Creatinine (0.5–1.3) (mg/dl) | |
| Minimum–maximum | 0.5–1.30 |
| Mean±SD | 0.80±0.22 |
| Median | 0.80 |
| Serum calcium (8.4–10.2) (mg/dl) | |
| Normal | 55 (91.7) |
| Abnormal | 5 (8.3) |
| Minimum–maximum | 8.0–9.20 |
| Mean±SD | 8.57±0.24 |
| Median | 8.60 |
| Ionized calcium (4.8–5.6) (mg/dl) | |
| Normal | 52 (86.7) |
| Abnormal | 8 (13.3) |
| Minimum–maximum | 4.40–8.60 |
| Mean±SD | 5.20±0.53 |
| Median | 5.20 |
| ESR (*n*<15) (mm/first hour) | |
| Minimum–maximum | 5.0–175.0 |

(*Continued*)

**Table 3 (*Continued*)**

| Variables | *n* (%) |
|---|---|
| Mean±SD | 64.19±55.63 |
| Median | 44.50 |
| Normal | 21 (35) |
| Abnormal | 39 (65) |
| CRP (0–6) (mg/l) | |
| Minimum–maximum | 0.0–100.0 |
| Mean±SD | 14.55±21.56 |
| Median | 5.25 |
| Normal | 34 (56.7) |
| Abnormal | 26 (43.3) |

ALT, alanine transaminase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PLT, platelet; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

**Table 4 Distribution of the studied cases according to some serological and immunological parameters (*n*=60)**

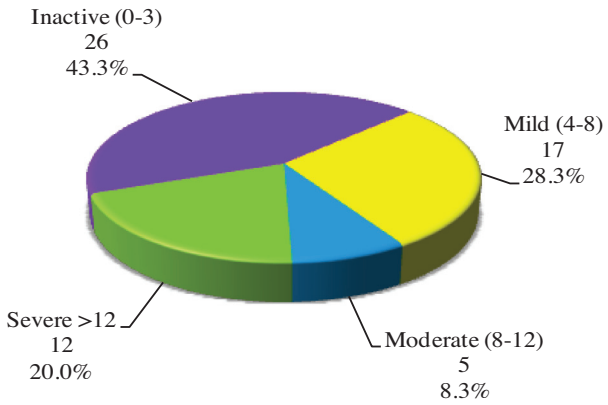
| Variables | *n* (%) |
|---|---|
| C4 (10–40) (mg/dl) | |
| Minimum–maximum | 0.06–62.0 |
| Mean±SD | 19.08±14.67 |
| Median | 15.50 |
| Normal (10.0–40.0) | 37 (61.7) |
| Abnormal | 23 (38.3) |
| C3 (90–180) (mg/dl) | |
| Minimum–maximum | 15.0–149.0 |
| Mean±SD | 76.11±37.75 |
| Median | 80.50 |
| Normal (90.0–180.0) | 26 (43.3) |
| Abnormal | 34 (56.7) |
| ANA (*N*<1/40) | |
| Minimum–maximum | 80.0–640.0 |
| Mean±SD | 286.33±182.62 |
| Median | 320.0 |
| Negative | 0 (0.0) |
| Positive | 60 (100.0) |
| Anti-ds-DNA (*N*≤75 IU/l) | |
| Minimum–maximum | 35.0–453.0 |
| Mean±SD | 159.88±93.99 |
| Median | 115.0 |
| Negative | 2 (3.3) |
| Positive | 58 (96.7) |

ANA, antinuclear antibody.

levels, and four patients (20.0%) had sufficient levels. In group I, the mean value of PTH was 36.52–12.13 pg/ml, whereas in group II it was 34.40±15.19 pg/ml. There was a statistically significant difference between group I (cases) and group II (controls) as regards 25(OH)D levels ($P$=0.033), with no statistically significant difference between the two groups ($P$=0.576) as regards PTH (Table 6 and Figs 2 and 3).

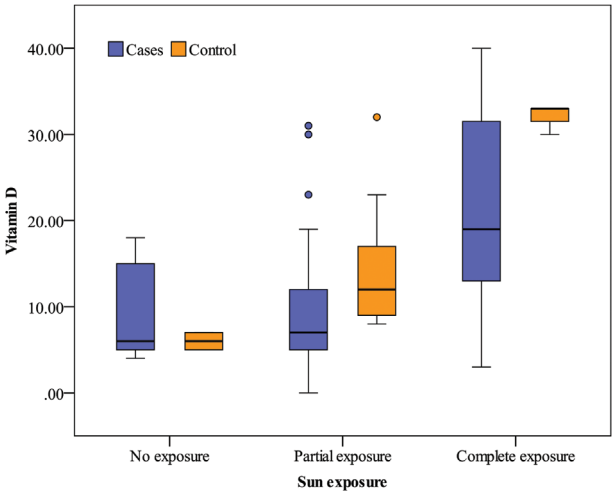
We studied the cases through a detailed questionnaire comprising details on dress style, outdoor activity, calcium supplementations, and vitamin D supplementations, and

**Figure 1**



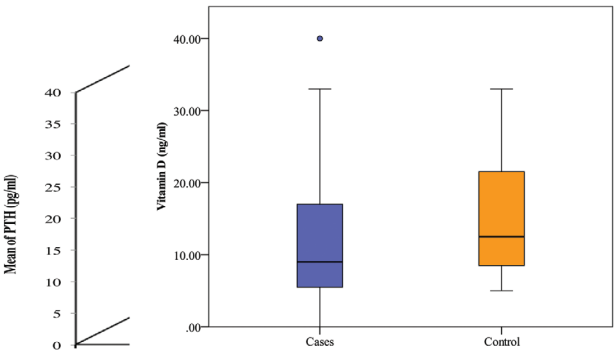

Distribution of the studied cases according to SLEDAI. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

**Figure 2**




Relation between 25(OH) D with sun exposure in each group.

**Figure 3**




Comparison between the two studied groups according to PTH. PTH, parathyroid hormone.

we found that there was a high statistically significant difference between groups I and II as regards outdoor activity ($^{FE}P$<0.001) and no statistically significant

**Table 5 Distribution of the studied cases according to SLEDAI (*n*=60)**

| Variable | *n* (%) |
|---|---|
| SLEDAI | |
| Inactive (0–3) | 26 (43.3) |
| Mild (4–8) | 17 (28.3) |
| Moderate (8–12) | 5 (8.4) |
| Severe>12 | 12 (20.0) |
| Minimum–maximum | 0.0–19.0 |
| Mean±SD | 6.63±5.74 |
| Median | 5.50 |

SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

**Table 6 Comparisonbetween the two studied groups according to 25(OH)D and PTH**

| Variables | Cases (*n*=60) [*n* (%)] | Control (*n*=20) [*n* (%)] | Test of significance | *P* |
|---|---|---|---|---|
| 25(OH)D (ng/ml) | | | | |
| Deficient<10 | 35 (58.3) | 8 (40.0) | $\chi^2$=2.157 | 0.340 |
| Insufficient 10–30 | 18 (30.0) | 8 (40.0) | | |
| Sufficient 30–100 | 7 (11.7) | 4 (20.0) | | |
| Minimum–maximum | 0.0–40.0 | 5.0–33.0 | *Z*=2.127* | 0.033* |
| Mean±SD | 12.08±9.41 | 15.90±9.44 | | |
| Median | 9.0 | 12.50 | | |
| PTH (1–65) (pg/ml) | | | | |
| Minimum–maximum | 11.0–60.0 | 10.0–55.0 | *t*=0.566 | 0.576 |
| Mean±SD | 36.52–12.13 | 34.40±15.19 | | |
| Median | 35.0 | 35.40 | | |

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
*Statistically significant at $P \leq 0.05$.

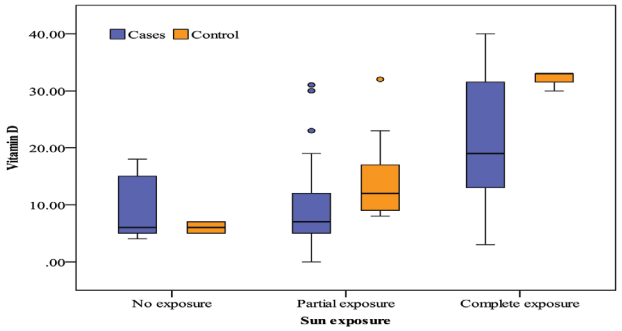
difference between the two groups as regards dress style ($^{MC}P$=0.917) (Table 7).

There was a significant correlation between 25(OH)D levels and sun exposure in group I (*P*=0.006) and group II (*P*=0.005).We also found a highly significant correlation between 25(OH)D levels and outdoor activity in group I (*P*≤0.001) and group II (*P*≤0.001) (Table 8 and Figs 4 and 5).

The mean value of 25(OH)D level in patients with no activity was 15.65±11.60 ng/ml; it was 12.06± 7.34 ng/ml in patients with mild activity, 6.20± 2.28 ng/ml in patients with moderate activity, and 6.83±3.61 ng/ml in patients with severe activity. There was a significant inverse correlation between vitamin $D_3$ levels and SLEDAI (*P*=0.043) (Table 9 and Fig. 6 and 7).

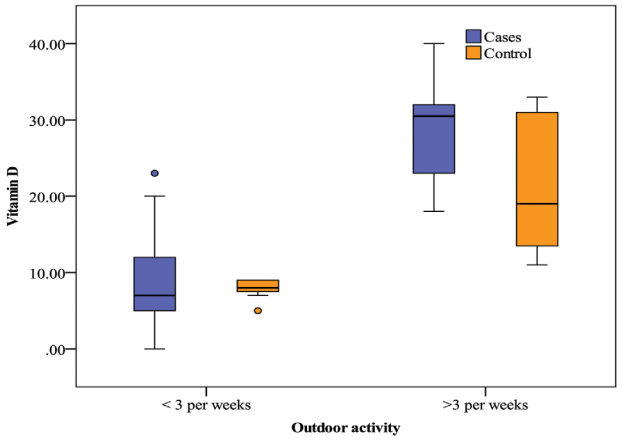
The mean value of 25(OH)D level in patients with no vitamin D supplementation was 7.51±4.15 ng/ml, whereas it was 25.80±7.08 ng/ml in patients with positive vitamin D supplementation. There was a high statistically significant correlation between 25 (OH)D levels and vitamin D supplementation (*P*<0.001).

**Figure 4**



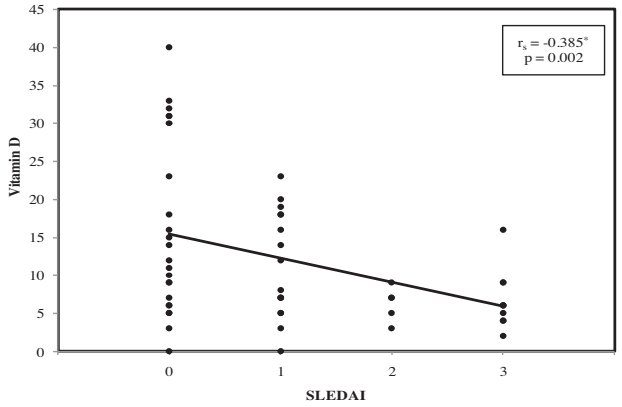

Relation between 25(OH)D and sun exposure in each group. 25(OH)D, 25-hydroxyvitamin D.

**Figure 5**




Relation between 25(OH)D with outdoor activity in each group. 25(OH)D, 25-hydroxyvitamin D.

**Figure 6**




Correlation between 25(OH)D and SLEDAI in the case group. 25(OH)D, 25-hydroxyvitamin D; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

**Table 7 Comparison between the two groups according to dress style and outdoor activity**

| Variables | Patients ($n$=60) [$n$ (%)] | Control ($n$=20) [$n$ (%)] | $\chi^2$ | $P$ |
|---|---|---|---|---|
| Sun exposure (dress style) | | | | |
| Minimal exposure | 6 (10.0) | 2 (10.0) | 0.253 | $^{MC}P$=0.917 |
| Partial exposure | 42 (70.0) | 15 (75.0) | | |
| Adequate exposure | 12 (20.0) | 3 (15.0) | | |
| Outdoor activity | | | | |
| ≤3 per week | 50 (83.3) | 8 (40.0) | 14.127* | $^{FE}P$<0.001* |
| >3 per week | 10 (16.7) | 12 (60.0) | | |
| Calcium supplements | 37 (61.7) | 0 (0.0) | – | – |
| Vitamin D supplements | 15 (25.0) | 0 (0.0) | – | – |

FE, Fischer's exact test; MC, Monte Carlo test. *Statistically significant at $P \leq 0.05$.

**Table 8 Relation between 25(OH)D with sun exposure and outdoor activity in each group**

| | $N$ | 25(OH)D | | | Test of significance | $P$ |
|---|---|---|---|---|---|---|
| | | Minimum–maximum | Mean±SD | Median | | |
| Sun exposure | | | | | | |
| Cases | | | | | | |
| No exposure | 6 | 4.0–18.0 | 9.0±5.97 | 6.0 | $^{KW}\chi^2$=10.370* | 0.006* |
| Partial exposure | 42 | 0.0–31.0 | 9.88±7.43 | 7.0 | | |
| Complete exposure | 12 | 3.0–40.0 | 21.33±11.64 | 19.0 | | |
| Control | | | | | | |
| No exposure | 2 | 5.0–7.0 | 6.0±1.41 | 6.0 | $^{KW}\chi^2$=10.497* | 0.005* |
| Partial exposure | 15 | 8.0–32.0 | 14.0±6.86 | 12.0 | | |
| Complete exposure | 3 | 30.0–33.0 | 32.0±1.73 | 33.0 | | |
| Outdoor activity | | | | | | |
| Cases | | | | | | |
| ≤3 per week | 50 | 0.0–23.0 | 8.68±5.34 | 7.0 | $Z$=4.874* | <0.001* |
| >3 per week | 10 | 18.0–40.0 | 29.10±6.23 | 30.50 | | |
| Control | | | | | | |
| ≤3 per week | 8 | 5.0–9.0 | 7.88±1.36 | 8.0 | 3.716* | <0.001* |
| >3 per week | 12 | 11.0–33.0 | 21.25±8.65 | 19.0 | | |

25(OH)D, 25-hydroxyvitamin D; KW, Kruskal–Wallis test. *Statistically significant at $P \leq 0.05$.

**Table 9 Relation between 25(OH)D with different parameters in the case group**

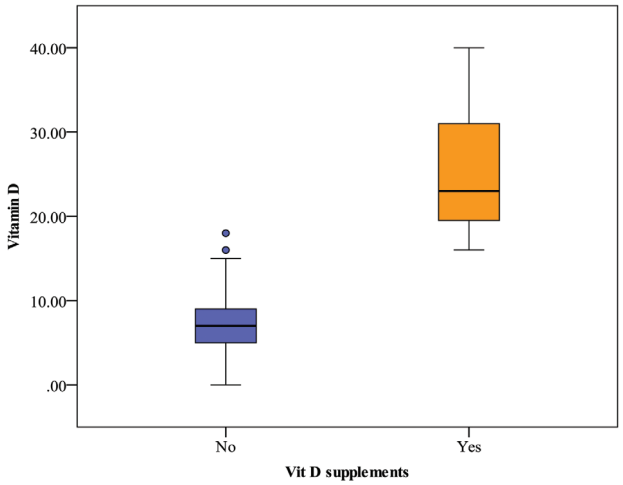
| | $N$ | 25(OH)D | | | Test of significance | $P$ |
|---|---|---|---|---|---|---|
| | | Minimum–maximum | Mean±SD | Median | | |
| SLEDAI | | | | | | |
| Inactive (0) | 26 | 0.0–40.0 | 15.65±11.60 | 11.50 | $^{KW}\chi^2$=8.158* | 0.043* |
| Mild (1) | 17 | 0.0–23.0 | 12.06±7.34 | 12.0 | | |
| Moderate (2) | 5 | 3.0–9.0 | 6.20±2.28 | 7.0 | | |
| Severe (3) | 12 | 2.0–16.0 | 6.83±3.61 | 6.0 | | |
| $r_s$ ($P$) | | | –0.385* (0.002*) | | | |
| Vitamin D supplements | | | | | | |
| No | 45 | 0.0–18.0 | 7.51±4.15 | 7.0 | $Z$=5.727* | <0.001* |
| Yes | 15 | 16.0–40.0 | 25.80±7.08 | 23.0 | | |
| Calcium supplements | | | | | | |
| No | 23 | 0.0–40.0 | 12.04±9.05 | 9.0 | $Z$=0.175 | 0.861 |
| Yes | 37 | 0.0–33.0 | 12.11±9.75 | 9.0 | | |

25(OH)D, 25-hydroxyvitamin D; KW, Kruskal–Wallis test; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index. *Statistically significant at $P \leq 0.05$.

The mean value of 25(OH)D level in patients with no calcium supplementation was 12.04±9.05 ng/ml, whereas it was 12.11±9.75 ng/ml in patients with positive calcium supplementation. There was no significant correlation between 25(OH)D levels and calcium supplementation ($P$=0.861).

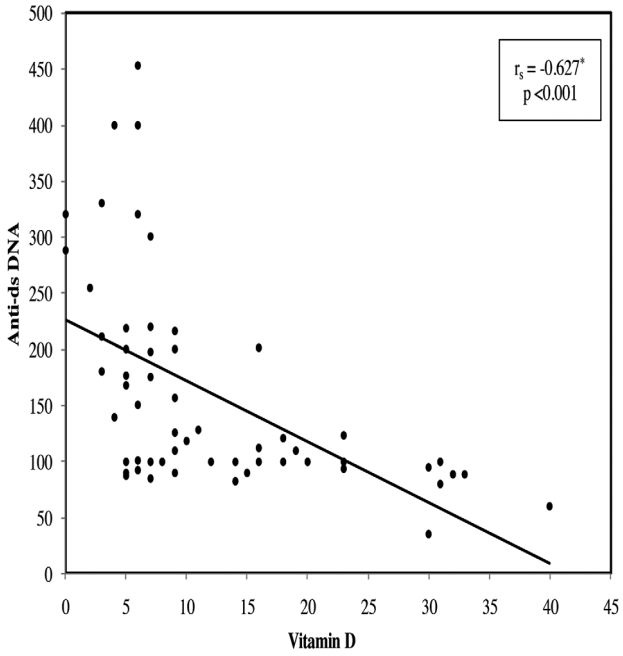
Serum 25(OH)D was inversely correlated to anti-ds-DNA ($P$<0.001) and ESR ($P$<0.001), but directly correlated to C3 ($P$=0.029) and C4 ($P$=0.002). There was no significant correlation with CRP ($P$=0.110) and serum ionized calcium ($P$=0.681) (Table 10 and Figs 8–11).

**Figure 7**




Relation between 25(OH)D with vitamin D supplements in the case group. 25(OH)D, 25-hydroxyvitamin D.

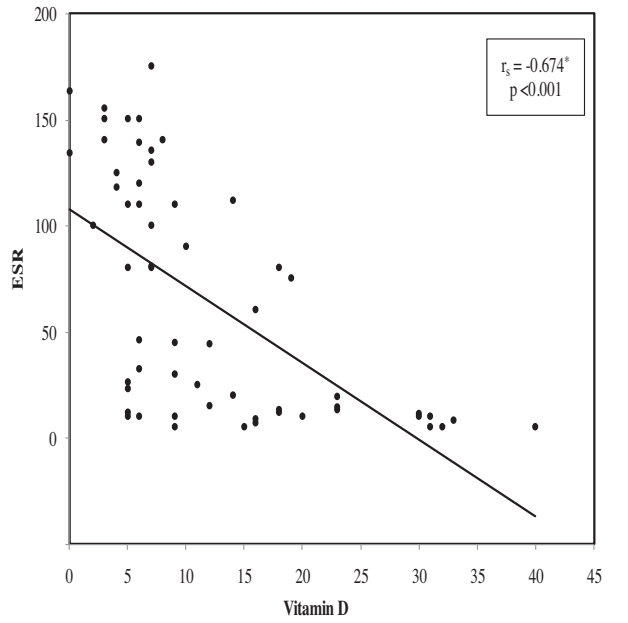
**Figure 8**




Correlation between 25(OH)D and SLE indices in the case group. 25 (OH)D, 25-hydroxyvitamin D; SLE, systemic lupus erythematosus.

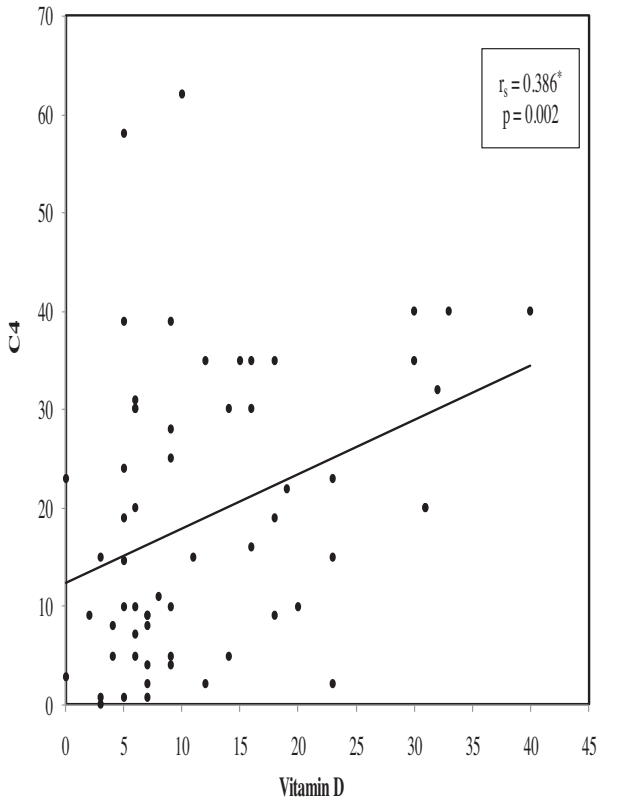
We considered patients to have positive vitamin D supplementations if they received vitamin D at a dose of 200 IU/day for the last 3 months. In group I, among the 15 patients (25.0%) who were receiving vitamin D supplementation, 8 (30.8%) had no activity and seven (41.2%) had mild activity; however, among the 45 patients (75%) who did not receive vitamin D supplementation, 18 (69.2%) had no activity, 10 (58.8%) had mild activity, five had moderate activity, and 12 had severe disease activity (Table 11). Consequently, there was a statistically significant relation between vitamin D supplementations and

**Figure 9**




Correlation between 25(OH)D and SLE indices in the case group. 25 (OH)D, 25-hydroxyvitamin D; SLE, systemic lupus erythematosus.

**Figure 10**




Correlation between 25(OH)D and SLE indices in the case group. 25 (OH)D, 25-hydroxyvitamin D; SLE, systemic lupus erythematosus.

SLE activity assessed using SLEDAI score ($^{MC}P$=0.030). Receiver operating characteristic curve of serum 25(OH)D (Fig. 5) showed that serum 25(OH)

**Table 10 Correlation between 25(OH)D and different parameters in the case group**

| | 25(OH)D | |
|---|---|---|
| | $r_s$ | *P* |
| Anti-ds-DNA | −0.627* | <0.001* |
| C4 | 0.386* | 0.002* |
| C3 | 0.282* | 0.029* |
| Ionized calcium | 0.054 | 0.681 |
| ESR | −0.674* | <0.001* |
| CRP | −0.208 | 0.110 |

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; 25 (OH)D, 25-hydroxyvitamin D. *Statistically significant at $P \leq 0.05$.

D can significantly discriminate between inactive or mild and moderate or severe SLE patients at cut-off level 'less than or equal to 9 ng/ml', with a sensitivity of 94.12% and specificity of 55.81% (Table 12 and Fig. 12).

## Discussion

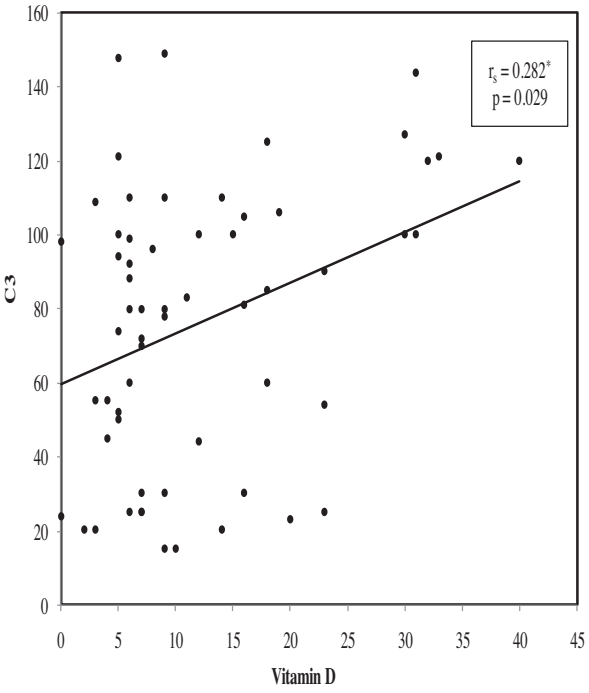
Vitamin D deficiency has been linked to classic cardiovascular risk factors such as type 2 diabetes mellitus, obesity, hypertension, and atherogenic dyslipidemia [16]. It has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency [17].

In the past few years, several reports pointed out a putative role for the active metabolite 1,25(OH)$D_3$ in immune system regulation, which lately was confirmed by data showing that macrophages and monocyte-derived DCs express the enzyme 25(OH)$D_3$ 1-α-hydroxylase (VD3 1A hydroxylase) also known as cytochrome p450 27B1 (CYP27B1). In this way, 1,25(OH)$D_3$ is generated locally and binds to vitamin D receptor in immune cells, thereby exerting concentration-dependent anti-inflammatory autocrine and paracrine effects in lymphoid microenvironments [7].

Recent data showed that vitamin D has an inhibitory effect on dendritic cells, CD4, CD8, B lymphocytes, and the production of cytokines, such as IFN, IL-2, IL-6, and TNF, and increases the number of T regulatory cells and synthesis of other cytokines such as IL-4, IL-10, and TGF [8].

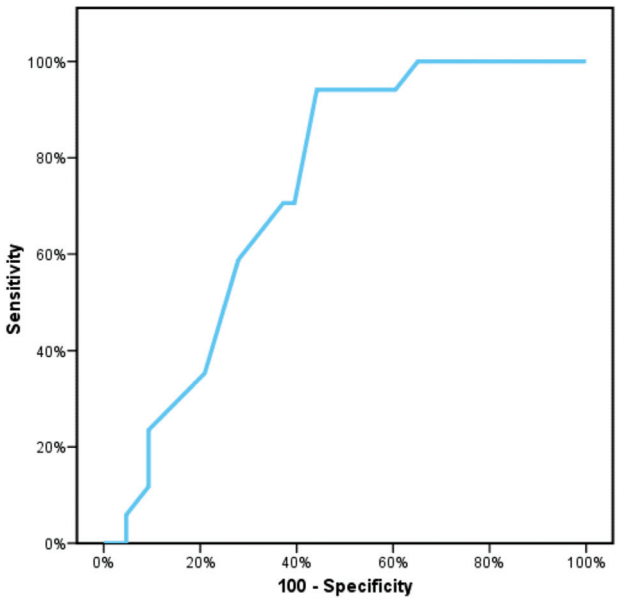
Recent epidemiological evidence showed a significant association between vitamin D deficiency and incidence of autoimmune diseases [18]. In addition, it was also found that lower levels of vitamin D were associated with higher disease activity in rheumatoid arthritis [19], undifferentiated connective tissue disease [20], multiple sclerosis [21,22], and inflammatory bowel disease. Vitamin D receptor gene polymorphism has been linked with SLE susceptibility in Asian, Polish,

**Figure 11**




Correlation between 25(OH)D and SLE indices in the case group. 25 (OH)D, 25-hydroxyvitamin D; SLE, systemic lupus erythematosus.

**Figure 12**




Receiver operating characteristic curve for 25(OH)D to predict (moderate or severe) cases. 25(OH)D, 25-hydroxyvitamin D.

and Egyptian patients [23]. Furthermore, an inverse correlation has been described between the supplementation of vitamin D and the development of type 1 diabetes mellitus [24].

According to our results, the prevalence of vitamin D deficiency 'less than 10 ng/ml' was detected in 58.3% of

**Table 11 Relation between vitamin D supplementation and SLE activity through SLEDAI score (*n*=60)**

| Variable | SLEDAI [*n* (%)] | | | | $\chi 2$ | $M_{CP}$ |
|---|---|---|---|---|---|---|
| | Inactive (0–3) (*n*=26) | Mild (4–8) (*n*=17) | Moderate (8–12) (*n*=5) | Severe>12 (*n*=12) | | |
| Vitamin D supplements | | | | | | |
| No | 18 (69.2) | 10 (58.8) | 5 (100.0) | 12 (100.0) | 8.353* | 0.030* |
| Yes | 8 (30.8) | 7 (41.2) | 0 (0.0) | 0 (0.0) | | |

MC, Monte Carlo test; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index. *Statistically significant at $P \leq 0.05$.

**Table 12 Agreement (sensitivity, specificity, and accuracy) for 25(OH)D to predict (moderate, severe) cases**

| | AUC | *P* | Cutoff | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|
| 25(OH)D | 0.733* | 0.005* | ≤9 | 94.12 | 55.81 | 45.7 | 96.0 |

AUC, area under the curve; 25(OH)D, 25-hydroxyvitamin D; NPV, negative predictive value; PPV, positive predictive value. *Statistically significant at $P \leq 0.05$.

patients and 40% of controls, and vitamin D insufficiency 'less than 30 ng/ml' was present in 30% of patients and 40% of controls.

Higher rates of vitamin D deficiency were observed in an Egyptian study status among university students in Zagazig; they found the prevalence of vitamin D insufficiency to be 74.6% and that of deficiency to be 28.5% [25]. Moreover, many studies on vitamin D were conducted in Arabic countries, with prevalence ranging from 50 to 100% according to the studied population and the cutoff point of vitamin D deficiency and insufficiency [26].

Fuleihan *et al.* [27] conducted a study in Lebanon on 465 women with conservative dress style, considering a cutoff of 20 ng/ml for vitamin D deficiency. They found the prevalence of vitamin D deficiency to be 95%. In Morocco, Allali *et al.* [28] performed the same study on 415 patients, considering a cutoff of 30 ng/ml for vitamin D deficiency, and the prevalence was 91%. In another study conducted by Al-Elq *et al.* [29], the prevalence of vitamin D deficiency in Kingdom of Saudi Arabia was 100 and 96%, respectively.

Furthermore, large population-based studies conducted in different parts of the world reveal a high prevalence of vitamin D deficiency and/or insufficiency. Nationwide surveys in Canada, including more than 5000 people, Korea, including more than 6000 people, and Australia, including more than 11 000 people, showed the prevalence of vitamin D deficiency in 62–67% of cases 'less than 30 ng/ml', 56% of cases 'less than 20 ng/ml', and 31% of cases 'less than 20 ng/ml', respectively [30].

The association between vitamin D and SLE was first described in 1979 [9]. Several studies worldwide have reported that vitamin D deficiency is more prevalent among SLE patients compared with the general population [10]. This can be attribute to the fact that patients with SLE are advised to avoid direct sunlight, a common trigger of disease flares and also the primary source of vitamin $D_3$. The risk for vitamin D deficiency is even higher among SLE patients compared with the general population [11]. In addition, other lupus-related factors that may contribute to vitamin D deficiency include renal disease [12] and the use of steroids that are thought to alter the metabolism of vitamin D [13]. Recent literature also showed that SLE patients produce antivitamin D antibodies [14].

In current study, we found a statistically significant difference between groups I and II as regards vitamin D levels. In group I, 58.3% of patients had deficient levels of vitamin D (<10 ng/ml), 30.0% had insufficient levels (10–30 ng/ml), and 11.7% of patients had sufficient levels (30–100 ng/ml). However, in group II, 40.0% of patients had deficient levels, 40.0% had insufficient levels, and 20.0% had sufficient levels.

Our results showed that vitamin D deficiency is highly prevalent in SLE patients compared with healthy controls, although the latter group shows relatively high prevalence of deficiency and insufficiency; this might be related, in Egypt, to the less outdoor activities and the dress style (Niqab or Islamic veil). This was also explained by Fragoso *et al.* [31] to be a result of modern life activities that prevents sun exposure and consequently reduces vitamin D synthesis. However, in our country, cultural behaviors may play the major role in nonexposure to sun, together with the socioeconomic factor that does not allow Egyptians to afford expensive food products rich in vitamin D such as Oily Fish (Smoked Salmon, Swordfish, Canned Trout, and Tuna), cod liver oil, mushrooms, fortified cereals, caviar, soymilk, and almond milk [32].

Our findings are in agreement with those of Damanhouri [33], who conducted a study on 165 SLE patients and 214 healthy controls and found that the prevalence of vitamin D insufficiency and deficiency in SLE patients was higher than that in the control group, wherein it was 98.8 versus 55% for the deficiency and 89.7 versus 20% for the insufficiency ($P<0.0001$).

Another study conducted by Kamen *et al.* [34] found lower 25(OH)D in 123 SLE patients when compared with 240 age and sex-matched controls. Furthermore, our results also confirmed those of Kim *et al.* [35], who found a significantly lower vitamin D level in SLE patients in comparison with healthy controls.

In contrast, Stockton *et al.* [36], who conducted their study on 24 SLE female patients and 21 healthy female controls, found that there was no significant difference in 25(OH)D levels between groups. The authors explained that this difference was because this study was conducted in Brisbane, Queensland, where ultraviolet radiation levels are high almost all year round; thus, the higher 25(OH)D levels may reflect inadequate photoprotection. It may also be because the mean SLEDAI score of the patients was 4.3, which means that they had mild disease activity. Because of the known effects of parathyroid hormone on calcium hemostasis and serum vitamin D levels as was explained before, we selectively chose our population (80 individuals) with normal serum PTH, which allowed us to study the relation between serum vitamin D levels and SLE activity more accurately. Moreover, as this study was concerned about dress style and the duration of sun exposure through outdoor activities, we considered patients who wore face veil (Niqab) to have minimal or poor sun exposure, women wearing head scarf (Islamic veil) to have partial sun exposure, and women not wearing the veil to have adequate sun exposure.

In group I, there were six patients (10%) with minimal or poor sun exposure, 42 patients (70.0%) with partial sun exposure, and 12 patients (20%) with adequate sun exposure; in group II, there were two individuals (10.0%) with minimal or poor sun exposure, 15 (75%) with partial sun exposure, and three (15.0%) with adequate sun exposure. Our results showed no statistically significant difference between the two groups as regards sun exposure ($^{MC}P=0.917$).

The results also showed a high statistically significant difference between groups I and II as regards outdoor activity ($^{FE}P<0.001$). Eventually, we studied the correlation of dress style and outdoor activities with serum vitamin D in both groups, and the results showed a significant positive correlation between the two parameters and serum vitamin D in the two groups ($P=0.006$ and $P<0.001$, respectively). These findings are in agreement with many studies that reported a high prevalence of vitamin D deficiency in winter due to decreased sun exposure [30,37,38], thus confirming the importance of frequent direct sun exposure on increasing serum vitamin D levels, in both the SLE patient group and normal healthy group. However, few reports from the gulf area reported a reversed seasonal effect, with a higher prevalence of vitamin D deficiency in summer. This was explained by avoidance of outdoor activity in the very hot and humid summer that may cause heat stroke, heat exhaustion, or mortality; however, outdoor activity is much more encouraged in the sunny warm winter [39].

In the present study, we evaluated patients for calcium supplementation, in which we considered patients to have positive supplementation, if they received a dose of 800 mg/day elemental calcium for the last 3 months. The results showed that 37 patients (61.7%) received calcium supplementations and 23 patients (38.3%) did not receive calcium supplementations. Furthermore, the results showed normal ionized calcium results in 86% of patients. Surprisingly, we did not find a significant correlation between serum vitamin D levels with either calcium supplementation ($P=0.861$), or ionized calcium levels ($P=0.681$). Thus, our results showed the interesting finding of the incomplete protection offered by treatment with oral calcium supplementation against vitamin D deficiency.

Our finding is in agreement with that of Rajalingham *et al.* [40], who studied the role of vitamin D in SLE; they also found that it is noteworthy that treatment with oral calcium did not completely protect against vitamin D deficiency. These results are supported by others [41].

In our study, we evaluated patients for vitamin D supplementation, in which we considered patients to have positive supplementation if they received a dose of 200 IU/day oral vitamin D for the last 3 months, and the results showed that 15 patients (25%) received supplementation and 45 patients (75%) did not receive. Moreover, a significant positive correlation was detected between serum vitamin D levels and vitamin D supplementations ($P<0.001$).

In the present work, we studied the relation between vitamin D supplementation and SLE activity assessed

using SLEDAI score in group I. The results showed that there was a significant statistical relation ($^{MC}P$=0.030) between them, concluding that vitamin D supplementation through its correcting effect on low serum vitamin D levels in SLE patients can significantly reduce the risk for high disease activity.

In accordance with our findings, Petri *et al.* [42] investigated the effects of vitamin D in 1006 patients over 128 weeks. On the first visit, 25(OH) D levels less than 40 ng/ml were found in 76% of patients, of whom 85% were African American. These patients received supplementation with 50 000 U of vitamin $D_2$ (ergocalciferol) weekly. Subsequent results showed modest but a significant reduction in the risk for high disease activity, associated with the increase in 25(OH)D levels in the subset of patients with low levels of vitamin D at the beginning of the study.

Similarly, our findings are in agreement with those of Abou-Raya *et al.* [43], who also randomized 267 SLE patients in a 2 : 1 ratio to receive either oral cholecalciferol 2000 IU/day or placebo. After 12 months, there was a significant reduction in the levels of proinflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α), and improvement in anti-ds-DNA, C4, hemostatic markers (fibrinogen and vonWillebrand factor), and disease activity scores in the treatment group compared with the placebo group. This could be explained by the recent data that proved that vitamin D has an inhibitory effect on dendritic cells, CD4, CD8, B lymphocytes, and the production of cytokines, such as IFN, IL-2, IL-6, IL-4, IL-10, TNF, and TGF [44–46], which play an important role in the pathogenesis of activity and disease flares in SLE.

In the current study, assessment of the disease activity in SLE patients was carried out by applying SLEDAI score. In group I, 43.3% had no disease activity, 28.3% had mild disease activity, 8.3% had moderate disease activity, and 20% of them had severe disease activity. When we studied the correlation between serum vitamin D concentration and disease activity measured using SLEDAI score, we found a statistically significant inverse correlation ($P$=0.043), $r_s$ ($P$)=–0.385 (0.002).

The results are in agreement with those of Mok *et al.* [45], who demonstrated a significant inverse relation between the levels of 25(OH)$D_3$ and SLE disease activity scores, in particular the SLEDAI subscores of active renal, musculoskeletal, and hematological disease, after adjustment for multiple variables that included demographic characteristics, disease duration, duration of sunshine at the time of venepuncture, and the use of medications such as calcium, vitamin D, and immunosuppressive agents.

Our results are also in accordance with those of Amital *et al.* [46], as they demonstrated a significant inverse relation between the degree of SLE activity and serum vitamin D concentration. Although the relation was weak, it was statistically significant, implying that vitamin D insufficiency, among other factors, probably contributes to the development of active disease in patients with SLE. Moreover, Ben-Zvi *et al.* [44] also found that vitamin D level correlated inversely with disease activity measured using the SLEDAI score ($r$=–0.234; $P$=0.002) in 198 SLE patients. Our findings are consistent with several other observational studies [47]. Nevertheless, some others failed to show a link [37,48]. Authors believed that the discrepancy was related to many factors such as sample size, seasonal variation in vitamin D levels, the proportion of studied participants with high disease activity and the distribution of disease activity in different organs, and the very low disease activity [37,48].

Several serological and immunological biomarkers have been used to assess disease activity in patients with SLE; these include serum C3 and C4, and anti-ds-DNA antibodies, and also phase reactants ESR and CRP. Moreover, results from group I showed that there were high statistically significant inverse correlations between serum vitamin D levels and anti-ds-DNA ($P$<0.001) and ESR ($P$<0.001). The results also showed that there was a direct statistically significant correlation of C3 ($P$=0.029) and C4 ($P$=0.002) levels with serum vitamin D in group I, but we found no correlation with CRP levels ($P$=0.110).

Our findings are in agreement with those of Fahmi *et al.* [49], who found that there was an inverse correlation with vitamin D level, anti-ds-DNA, and ESR, whereas there was a direct correlation with vitamin D level and C3 in the disease activity group.

In accordance with our findings, Mok *et al.* [50], in their study on 290 SLE patients, found that 25 (OH)$D_3$ level correlated inversely and significantly with clinical SLE activity and anti-ds-DNA titers. Mandal *et al.* [47] also demonstrated a

significant inverse correlation between vitamin D level and ESR at baseline and in multiple linear regression analysis. In contrast, Attar *et al.* [51] found a positive correlation between 25(OH)D and C4 levels but not between the 25(OH)D and C3 levels. A similar finding was reported in a study conducted on 177 patients with SLE [52]. In addition, Suzan also found that low levels of C3 and C4 were strong predictors for 25(OH)D deficiency in lupus patients. This could be explained by the fact that the classical pathway is the dominant pathway in complement activation in SLE patients, and so the level of C4 is always low, whereas C3 may be either normal or lower than normal [53].

Moreover, in our observational study, we studied the relation between serum vitamin D level and different SLE symptoms. Although our study did not find a significant correlation to symptoms, results were the highest with fever and malaise ($P$=0.091), which are nonspecific features.

In a review of recent literature, many studies reported a negative correlation between vitamin D levels and fatigue symptom in SLE patients. This was confirmed by Guillermo Ruiz *et al.* [48], who in an observational longitudinal study found that changes in serum 25(OH)D levels were inversely associated with fatigue, as measured using a 0–10 visual analog scale. Ruiz-Irastorza *et al.* [41] also supports the same results. Finally, receiver operating characteristic curve for serum vitamin D in our study showed that serum vitamin D can significantly discriminate between inactive or mild and moderate or severe SLE patients at a cut-off level less than or equal to 9 ng/ml, with a sensitivity of 94.12% and specificity of 55.81%.

Thus, we can conclude that vitamin D deficiency is highly prevalent in active SLE patients than in healthy controls. 25(OH)D level correlates inversely with disease activity, which suggests an important role of vitamin $D_3$ in the pathogenesis of disease activity and flares. Finally, we conclude that oral calcium supplementation at a dose of 800 mg/day for the last 3 months offers incomplete protection against vitamin D deficiency, whereas vitamin D supplementation at a dose of 200 mg/day for the last 3 months offers a modest but a significant reduction in the risk for high disease activity.

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**Conflicts of interest**
There was no conflict of interest.

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