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Reduced serum concentrations of 25-hydroxy vitamin D in Egyptian patients with systemic lupus erythematosus: Relation to disease activity

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Recently, vitamin D deficiency has been implicated as a potential environmental factor triggering some autoimmune disorders, including systemic lupus erythematosus (SLE)). In addition, patients with SLE, especially those with increased disease activity, were suggested to have decreased vitamin D level, suggesting that vitamin D might play a role in regulating autoantibody production.

Material/Methods: To assess 25 hydroxy vitamin D [25(OH)D] status in Egyptian patients with SLE and its relation to disease activity. Clinical evaluation and assay of serum 25(OH)D, total calcium, phosphorous, alkaline phosphatase (ALP) and parathyroid hormone (PTH) were done on 60 SLE patients in comparison to 60 matched-healthy subjects. Serum 25(OH)D levels <30 and 10 ng/ml were defined as vitamin D insufficiency and deficiency, respectively.

Results: Serum 25(OH)D was significantly lower in patients than in controls (26.33±12.05 *vs.* 42.66±9.20 respectively, *p<0.0001),* with 13.30% and 60% being deficient and insufficient, respectively. Serum 25(OH)D levels were lower with increased disease activity *(p=0.03)* and frequency of photosensitivity *(p=0.02)* and photoprotection *(p=0.002).* Systemic lupus erythematosus disease activity index (SLEDAI) score (OR: 2.72, 95% CI: 1.42–5.18, *P=0.002*), photosensitivity (OR: 3.6, 95% CI: 1.9–6.8, *P<0.01*) and photoprotection (OR: 6.7, 95% CI: 2.9–8.8, *P<0.001*) were significant predictors of 25(OH)D level among SLE cases.

Conclusions: Low vitamin D status is prevalent in Egyptian SLE patients despite plentiful exposure to sunlight throughout the year, and its level is negatively correlated to disease activity. Future studies looking at a potential role of vitamin D in the pathophysiology and treatment of SLE are warranted.

key words: **Egyptian • 25 hydroxy vitamin D • photosensitivity • systemic lupus erythematosus**

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Background

Vitamin D is the common denominator of a group of sterols with a crucial role in phospho-calcic metabolism. The main source of vitamin D is the conversion of 7-dehydrocholesterol to pre-vitamin D_3 in the skin, by means of solar ultraviolet B radiation, and a lesser amount of vitamin D is obtained from food. Vitamin D_3 undergoes a 25-hydroxylation in the liver, with the resulting product, 25(OH)D or calcidiol, being the main circulating form of vitamin D. 25(OH)D levels are therefore used to determine the vitamin D status of a given individual [1]. The fully active form, 1, 25 dihydroxy vitamin D3 (1, 25 $(OH)_2 D_3$), is synthesized in the kidneys by the 25(OH) vitamin D-1α hydroxylase, an enzyme which is mainly induced by PTH. The main metabolic effect of 1, 25 $(OH)_2 D_3$, which is mediated through the interaction with vitamin D receptors (VDRs), is promoting the intestinal absorption and renal resorption of calcium in order to increase its circulating levels. Deficient levels of vitamin D promote PTH synthesis that results in bone resorption. Long-lasting depletion of vitamin D causes rickets and osteomalacia, with skeletal deformities in children and bone pain and increased risk of fractures in adults, respectively [2].

SLE is a chronic systemic autoimmune disease of unpredictable course and prognosis. Several studies have shown that ethnicity plays a vital role in determining the clinical features and disease outcome in patients with SLE [3]. Although the cause remains uncertain, several hereditary and environmental factors have been postulated to play a role in the development of SLE [4]. Recently, vitamin D deficiency has been implicated as a potential environmental factor triggering some autoimmune disorders, including SLE, since several immunoregulatory activities for 1, 25$(OH)_2 D_3$ have been identified [5]. In addition, it has been suggested that patients with SLE, especially those with increased disease activity, have decreased vitamin D level, indicating that vitamin D might play a role in regulating autoantibody production [6]. The prevalence of 25(OH)D is high in countries in the Middle East [7].

With this background we were stimulated to assess 25(OH) D status in Egyptian patients with SLE and its relation to disease activity.

Material and Methods

Study population and blood samples

This cross sectional case-control study was conducted on 60 Egyptian SLE patients (52 females and 8 males) recruited from the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University, Cairo, Egypt during the period from June 2008 to December 2009. Their ages ranged between 6–19 years, with a mean ±SD of 12.83±3.05 years. Patients fulfilled the criteria for diagnosis of SLE according to the 1982 revised American Rheumatism Association criteria [8]. All patients were positive for antinuclear antibodies (ANA) and antibody to double-stranded DNA antigen (anti-dsDNA). Patients were studied in comparison to 60 healthy age- and sex-matched Egyptian subjects serving as controls who had no clinical findings suggesting immunological or bone diseases. They were recruited from the outpatient clinic of the same hospital. They were 50 females and 10 males whose ages ranged between 7.2-18.5 years, with a mean ±SD of 13.10±4.21 years. All studied subjects had normal liver and kidney function tests; those with abnormal liver or kidney functions (based on serum alanine amino transferase and aspartate amino transferase; and serum creatinine) were excluded from the study. Also, all studied subjects had not received calcium and/or vitamin D therapy in the past 6 months.

Four milliliters of venous peripheral blood were collected from each patient and control subject under complete aseptic conditions, withdrawn into plain tubes, left to clot for 30 minutes and separated. Serum was divided into 2 portions, 1 for direct assay of total serum calcium, phosphorus and ALP, and the other portion was stored at –20°C for assay of 25(OH)D.

A written informed consent of participation in the study was signed by the parents or the legal guardians of the studied subjects. This study was approved by the Bioethical Research Committee, Faculty of Medicine, Ain Shams University Hospitals, Cairo, Egypt.

Study measurements

Patients' characteristics and clinical evaluation of SLE patients

The following variables were recorded at the time of inclusion: age, sex, disease duration, history of photosensitivity, current use of photoprotection (defined as the avoidance of excessive exposure of skin to the sun causing unwanted skin effects induced by ultraviolet rays, which is established through physical measures such as protective clothing, and daily application of broad-spectrum sunscreens [2]), hours of sun exposure per week, long bone fractures and current treatment (prednisone and its dose and duration, hydroxychloroquine (HCQ), immunosuppressive drugs). In addition, disease activity was assessed using SLEDAI score [9]. The original SLEDAI is a weighted, cumulative index of lupus disease activity. The total score falls between 0 and 105, with higher scores representing increased disease activity. The SLEDAI has been shown to be a valid and reliable disease activity measure in multiple patient groups, and has also has been shown to be sensitive to changes in disease activity in children. Increase in SLEDAI by ⩾3 indicates persistently active disease or flare-up [9].

Laboratory investigations (for patients and controls)

- Assay of serum 25(OH)D by enzyme-linked immunosorbent assay (ELISA) using a competitive protein binding assay kit for the measurement of 25(OH)D, which is based on the competition of 25(OH)D present in the sample with 25(OH) vitamin tracer, for the binding pocket of vitamin D protein (VDBP, Gc-globulin). According to current recommendations, serum 25(OH)D levels <30 and 10 ng/ml were defined as vitamin D insufficiency and vitamin D deficiency, respectively, while levels >30 ng/ml were defined as vitamin D sufficiency [10].

- Assay of total serum calcium by spectrophotometer using a Hitachi 917 autoanalyzer and Roch reagents. A calcium binding dye, Orthocresolphthalein complexone,

was used, which changes its color when binding to calcium. The intensity of the formed color is directly proportional to the concentration of calcium in the sample, and the absorbance of the formed color was monitored at 650 nm. A reference range of 8.5–10.5 mg/dl was used [11].

- Assay of serum inorganic phosphorous using a Hitachi 917 autoanalyzer and Roche reagents. The method is based on the reaction of phosphate ions with ammonium molybdate to form phosphomolybdate complex, which is colorless and measured directly by UV absorbance at 340 nm. A reference range of 3.5–5.5 mg/dl was used [12].

- Assay of serum ALP by spectrophotometer in which ALP activity was measured by the IFCC-recommended method using colorless 4-nitrophenyl phosphate (4-NP) as a substrate. A reference range of 50–140 IU/L was used [11].

- Assay of serum PTH was done by Immulite 2000 Intact PTH (Siemens Diagnostics, Los Angeles, CA, USA), a solid-phase, 2-site chemoluminescent enzyme-labelled immunometric assay with a reference range of 11-62 pg/ml [13].

- Estimation of ANA by indirect immuno-fluorescence antibody technique using the standard immuno-fluorescence on HEp-2 human epithelial cells (IMMCO Diagnostic Inc., Buffalo, NY, USA). Samples were considered positive if nuclear or cytoplasmic staining was positive at a dilution of ⩾1:80.

- Estimation of anti-dsDNA by standard ELISA kits (Immulisa, IMMCO Diagnostic, Inc., Buffalo, NY, USA).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, Version 16.0 (SPSS Inc, IL, USA). Description of quantitative variables was in the form of mean ±SD and range. Description of qualitative variables was in the form of frequency and percentage. Chi-Square test was used to compare frequency of qualitative variables among the different groups. Student's T test was used to assess the statistical significance of the difference between 2 population means in a study involving independent samples. ANOVA (analysis of variance) was used to test the difference in mean values of some parameters among multiple groups; when these were significant, a post-hoc test was performed to compare each 2 groups separately. Spearman's correlation test was used for correlating non-parametric variables. Risk estimation was done by using the odds ratio (OR), and a 95% confidence interval (CI) for the odds ratio was also calculated. If the value of 1 is not in the range of confidence interval, it can be concluded that there is a relative risk in 1 group compared to the other. Probability values (p) <0.05 were considered as significant.

Results

SLE patients and control subjects

Clinical and laboratory data and drug treatment of the study groups are summarized in Table 1.

Serum 25(OH)D levels in SLE patients

The mean serum 25(OH)D was significantly lower in SLE cases than controls ($p<0.0001$, Table 1), while the mean serum ALP was significantly higher in SLE cases ($p=0.04$). On the other hand, serum calcium, phosphorous and PTH did not significantly differ between both groups ($p>0.05$, Table 1).

Of the 60 studied SLE patients, 44 (73.30%) had low 25(OH) D levels, [8 (13.30%) were 25(OH)D deficient, 36 (60%) were insufficient], and 16 (26.70%) had normal 25(OH) D levels.

Lower serum 25(OH)D levels were significantly associated with higher SLEDAI scores ($p=0.03$, Table 2), with significant negative correlations between SLEDAI score and each of 25(OH)D levels ($r=-0.91$, $p<0.01$) and serum calcium ($r=-0.87$, $p<0.01$) and a significant positive correlation with PTH ($r=0.83$, $p=0.02$). Moreover, there was increased frequency of long bone fractures as 25(OH)D levels decreased ($p=0.003$). On the other hand, vitamin D status did not significantly differ according to age and disease duration ($p>0.05$, Table 2).

Effect of photosensitivity and sun exposure on 25(OH) D levels

Of the 38 cases with photosensitivity, 32 (84.21%) had low 25(OH)D status [8 (21.05%) were deficient and 24 (63.16%) were insufficient] and only 6 (15.79%) were 25(OH)D sufficient. Among the 44 cases on current photoprotection, 42 (95.45%) had low 25(OH)D status [8 (18.18%) were deficient and 34 (77.27%) were insufficient] and only 2 (4.55%) was 25(OH)D sufficient. In addition, serum 25(OH)D levels were significantly lower as frequency of photosensitivity ($p=0.02$) and photoprotection ($p=0.002$) increased (Table 2). In addition, SLEDAI score was significantly higher among cases on current photoprotection compared to those without (33 *vs.* 12, p<0.01). The mean duration of sun exposure among studied cases did not differ from controls [3.34±1.5 (1.8–4.9) *vs.* 4.23±2.48 (2.1–6.4) hours/week] in spite of being lower among cases ($p<0.05$). In addition, 25(OH)D levels did not differ according to duration of sun exposure ($p>0.05$, Table 2).

Effect of SLE therapy on 25(OH)D status

As shown in Table 3, significantly lower 25(OH)D levels were encountered as the dose ($p=0.03$) and duration ($p=0.04$) of prednisone increased. Also, significant negative correlations were detected between 25(OH)D level and each of dose ($r=-0.74$, $p=0.02$) and duration of prednisone ($r=-0.86$, $p=0.04$). On the other hand, serum 25(OH)D levels did not differ in relation to the frequency of use of immunosuppressives ($p>0.05$), while higher 25(OH)D levels were encountered as the frequency of use of HCQ increased ($p=0.04$).

Relation between 25(OH)D level and other laboratory bone parameters

Significantly lower calcium, and higher ALP and PTH were detected as 25(OH)D levels decreased ($p=0.0001$ in all), whereas a non-significant difference was detected regarding serum phosphorous ($p>0.05$). On using post-hoc tests,

Table 1. Characteristics of SLE patients and controls.

	SLE (n=60)	Controls (n=60)
– Sex (female/male)	52/8	50/10
– Age, years, mean ±SD	12.83±3.05	13.10±4.21
Range	6–19	7.2–18.5
– Duration of diagnosis, months, mean ±SD	13.63±9.361	NA
Range	3–36	
– SLEDAI score, mean ±SD	14.33±11.03	NA
Range	0–42	
Patients, no. (%) 0	14 (23.34)	NA
1–3	20 (33.33)	
>3	26 (43.33)	
– Current photosensitivity, no. (%)	38 (63.33)	NA
– Current photoprotection, no. (%)	44 (73.33)	0 (0)
– Duration of sun exposure, hours/week		
Mean ±SD	2.34±1.5	4.23±2.48
Range	0.5–3.7	2.1–6.4
– Long bone fractures, no. (%)	4 (6.66)	0 (0)
– Treatment with prednisone, no. (%)	60 (100)	NA
Daily dose, mg/day, mean ±SD	31±14.93	NA
Range	10–60	
Duration, months, mean ±SD	10.87±7.20	NA
Range	3–33	
– Treatment with HCQ		
Patients, no. (%)	26 (43.33)	NA
– Treatment with immunosuppressives		
Patients, no. (%)	28 (46.66)	NA
– Treatment with calcium & vitamin D		
Patients, no. (%)	0 (0)	0 (0)
– Serum 25(OH)D, ng/dl, mean ±SD	26.33±12.05	42.66±9.20
Range	8–50	36–58
– Serum calcium, mg/dl, mean ±SD	8.93±0.75	9.53±0.53
Range	7.80–10.20	8.9–10.40
– Serum P, mg/dl, mean ±SD	3.82±0.66	4.56±0.83
Range	1.8–5.5	3.8–5.4
– Serum ALP, IU/L, mean±SD	113.93±36.92	91.26±9.43
Range	89.5–195	81–120
– Serum PTH, pg/ml, mean ±SD	54.23±23.86	48.8±9.08
Range	33–110	29–59

Results are expressed as mean±SD and range, frequency and percentage. NA – non applicable; SLE – systemic lupus erythematosus; SLEDAI – systemic lupus erythematosus disease activity index; HCQ – hydroxychloroquine; 25(OH)D – 25 hydroxy vitamin D; P – phosphorous; ALP – alkaline phosphatase; PTH – parathyroid hormone.

Table 2. Clinical and laboratory variables of SLE cases by 25(OH)D status.

		25 (OH) Vitamin D level (ng/ml)			F/x2	P
		<10	10–30	>30		
– Number		8	36	16		
– Age, years						
Mean±SD		13.63±1.59	12.89±3.42	11±3.36	0.99	0.38
Range		11–16	7–19	6–13		
– Duration of diagnosis, months						
Mean ±SD		21±11.48	13.55±10.21	10.12±2.8	1.91	0.16
Range		12–36	3–36	5–12		
– SLEDAI score						
Patients, no. (%)	1–3	0 (0%)	4 (11.11)	10 (62.5)	10.38	0.03*
	>3	8 (100)	32 (88.89)	6 (37.5)		
– Photosensitivity						
Positive, no (%)		8 (100)	24 (66.66)	6 (37.5)	6.11	0.02*
Negative, no (%)		0(0)	12 (33.34)	10 (62.5)		
– Current photoprotection						
Positive, no (%)		8(100)	34 (94.44)	2 (12.5)	12.35	0.002**
Negative, no (%)		0 (0)	2 (5.56)	14 (87.5)		
– Duration of sun exposure						
Hours/week, mean ±SD		2.1±0.47	2.3±0.52	3.6±1.4	1.56	0.43
Range		1.8–2.71	2.0–3.4	2.2–4.9		
– Long bone fractures						
Positive, no (%)		3 (37.5)	1 (2.8)	0 (0)	11.48	0.003**
Negative, no (%)		5 (62.5)	35 (97.2)	16 (100)		
– Serum calcium, mg/dl						
Mean ±SD		7.9±0.08	8.86±0.47	9.70±0.42	39.69	0.0001***
Range		7.8–8.1	8.2–9.4	8.9–10.2		
– Serum P, mg/dl						
Mean		1.97±0.17	3.85±0.47	4.18±7.45	0.57	0.57
Range		1.8–2.2	2.3–4.6	3.4–5.5		
– Serum ALP, IU/L		168.75±23.93	123±34.7	98.62±5.12	10.35	0.0001***
Range		135–195	94–190	89.5–112.2		
– PTH, pg/ml						
Mean ±SD		104.5±4.2	58.7±5.5	41.5±6.27	225.01	0.0001***
Range		100–110	41–69	33–52		

Results are expressed as mean±SD and range, frequency and percentage, *p<0.05, **p<0.01, ***p<0.001. 25(OH)D – 25 hydroxy vitamin D; SLEDAI – systemic lupus erythematosus disease activity index; P – phosphorous; ALP – alkaline phosphatase; PTH – parathyroid hormone.

regarding serum calcium, there were highly significant differences on comparing each group to the other *(p<0.001)*, as was the case with PTH *(p<0.001)*. Regarding ALP, there was a highly significant difference between 25(OH)D <10 ng/ml and 25(OH)D >30 ng/ml (*p<0.01*), 25(OH)D <10 ng/ml and 25(OH)D 10–30 ng/ml *(p=0.02)*, whereas a non-significant difference between 25(OH)D10–30 ng/ml and 25(OH)D >30 ng/ml groups was detected *(p>0.05)*.

Table 3. Effect of therapy given to SLE cases on 25(OH) D status.

	25 (OH) Vitamin D level (ng/ml)			F/x2	P
	<10	10–30	>30		
– Number	8	36	16		
– Treatment with prednisone	8–33	4–28	3–9		
Daily dose, mg/day					
Mean ±SD	45±10.14	30±13.51	20±4.34	5.69	0.03*
Range	20–60	15–50	10–25		
Duration, months					
Mean ±SD	21.75±8.44	13.38±6.98	6.3±2.26	4.54	0.04*
Range	8–33	4–28	3–9		
– Treatment with HCQ					
Positive, no (%)	2 (25)	14 (38.89)	10 (62.5)	4.69	0.04*
Negative, no (%)	6 (75)	22 (61.11)	6 (37.5)		
– Treatment with immunosuppressives					
Positive, no. (%)	4 (50)	16 (44.45)	8 (0)	1.36	0.42
Negative, no (%)	4 (50)	20 (55.55)	8 (100)		

Results are expressed as mean±SD and range, frequency and percentage, * p<0.05, ** p<0.01. SLE – systemic lupus erythematosus; HCQ – hydroxychloroquine.

Predictors of 25(OH)D level among SLE cases

In the current study, we studied the clinical predictors (age, sex, disease duration, SLEDAI score, photosensitivity, photoprotection, long bone fractures) and laboratory predictors (serum calcium, phosphorous, ALP and PTH) of 25 (OH) D level among SLE patients. SLEDAI score (OR: 2.72, 95% CI: 1.42–5.18, *P=0.002*), photosensitivity (OR: 3.6, 95% CI: 1.9–6.8, *P<0.01*) and photoprotection (OR: 6.7, 95% CI: 2.9–8.8, *P<0.001*) were significant predictors of 25 (OH)D level among SLE cases. Other clinical and laboratory parameters were non-significant predictors *(p>0.05).*

Discussion

Recently, vitamin D deficiency has been implicated as a potential environmental factor triggering some autoimmune disorders, including SLE, since several immunoregulatory activities for vitamin D have been identified [5]. Patients with SLE have multiple risk factors for vitamin D deficiency and the disease severity seems to be correlated with lower 25(OH)D serum levels. Therefore, it is important to consider the possibility of vitamin D deficiency in SLE patients [14].

The current study provides insights into vitamin D status and SLE among Egyptian patients. In this study, we have confirmed significantly lower 25(OH)D levels among SLE cases in addition to the finding that low 25(OH)D status is frequent in Egyptian lupus patients, since 73.30% of our cases had low 25(OH)D levels, with 60% being insufficient (<30 ng/ml) and 13.30% being deficient (<10 ng/ml) despite the fact that our population resides in areas with plenty of sunny days. A higher prevalence of low 25(OH) D levels [90% (75% insufficient, 15% deficient)] was detected in a Spanish study by Ruiz-Irastorza et al [15]. In addition, Kamen et al [16] found lower 25(OH)D levels in their African-American and Caucasian lupus patients compared to controls, but the prevalence of low 25(OH)D levels was slightly higher than ours [84.58% (66.7% insufficient, 17.88% deficient)]. A strikingly higher prevalence of low 25(OH)D status (98.8%) was detected in a recent study by Damanhouri [17] done at King Abdul Aziz University Hospital in Jedah, where 89.7% of their SLE patients had deficient levels and 9.1% had insufficient levels. On the other hand, lower prevalence rates were reported in the USA (65%) [18] and Canada (56%) [19].

Moreover, Sheng and associates [20] found significantly lower 25(OH)D and $1,25(OH)_2D_3$ levels in SLE patients compared to rheumatoid arthritis patients and normal controls in a study performed in Shanghai, while Muller et al. [21] found significantly lower 25(OH)D levels in SLE patients compared to osteoarthritis patients and normal controls in a study performed in Copenhagen, Denmark. In contradiction to our results, Redlich et al. [22] did not find a significant difference in 25(OH)D levels between SLE patients and controls, which could be due to the fact that they studied only patients with mild activity. The reasons behind the discrepancy between the findings of various studies are uncertain. Recent reports have stressed on methodological considerations in assay, and inter-laboratory variations, even when using the same assay procedures. Efforts to standardize assays and to improve accuracy and reproducibility have been recommended [2]. In addition, genetic susceptibility to vitamin D deficiency and ethnic variations could be other possible explanations [3].

Vitamin D is the common denominator of a group of sterols, with a crucial role in phospho-calcic metabolism. The major source of vitamin D is exposure to sunlight, but few foods naturally contain vitamin D. It has been suggested that 5–30 minutes of sun exposure between 10 am and 3 pm at least twice a week to the face, arms, legs, or back without sunscreen usually leads to sufficient vitamin D synthesis and that sunscreens with a sun protection factor of 8 or more appear to block vitamin D- producing ultraviolet rays [2].

Low 25(OH)D levels among SLE patients may be due to the fact that patients with SLE are frequently photosensitive and frequently use very high ultraviolet photoprotection [15,17,21]. This was confirmed in our study by the fact that serum 25(OH)D levels were significantly lower as frequency of photosensitivity and photoprotection increased together with the non-significant difference in the hours of sun exposure between SLE cases and controls. Other contributing factors preventing direct sunlight exposure include darker skin pigment, limited amount of vitamin D obtained from dietary sources [16], and cultural and religious practice of wearing clothes that cover the entire body (veiled and unveiled women) [17]. Carvalho et al [23] suggested another cause to explain the low vitamin D levels in SLE patients – the presence of anti-vitamin D antibodies in patients with SLE and other autoimmune diseases and the association of these autoantibodies with anti-dsDNA antibodies in SLE patients. Chronic use of corticosteroids and the deterioration of kidney functions in some SLE patients are other suggested causes [15]. SLE-related renal involvement may inhibit the conversion of 25(OH)D in the kidney to its biologically active form of $1,25(OH)_2D_3$ via inhibition of 1-α hydroxylase [2]. This risk factor was ruled out, since all patients in this study were free from kidney and liver dysfunctions.

Finally, an immunomodulatory effect of vitamin D may be involved in the pathogenesis of SLE. Vitamin D has a suppressive effect on the differentiation of DCs and T-helper (Th) 1 CD4 + T cells, leading to suppression of autoimmune disease. Additional mechanisms of vitamin D to suppress autoimmunity include increased T regulatory cells, decreased autoantibody production, diminished inflammatory mediator release, and perhaps tolerance reestablishment [24]. Also, vitamin D was found to affect lupus B-cell function directly [25]. The pleiotropic effects of vitamin D are mediated through its binding to the VDR. Few studies have correlated polymorphisms in the VDR gene with increased susceptibility to lupus [26,27].

A recent advance in the understanding of lupus pathogenesis is recognition of the role of interferon. The overexpression of interferon-responsive genes seen in active lupus patients is termed the interferon-α signature [28]. Activated plasmacytoid DCs are the primary source of interferon-α. Observations that $1,25(OH)_2D_3$ inhibits *in vitro* DC maturation/activation and type I interferon production suggest that giving vitamin D as a therapeutic intervention may be beneficial in lupus patients [29]. In animal models, vitamin D has already been suggested to be an effective treatment for SLE [30].

Reasons to prevent vitamin D deficiency in all patients, particularly those with lupus, are numerous. Bone density and muscle strength are often compromised by not only the frequent use of corticosteroids for disease suppression but also by disease activity itself [28]. The benefits of vitamin D in the prevention of growth retardation and rickets in children and osteomalacia in adults have been well described [1]. Recently, several randomized controlled trials have demonstrated that vitamin D supplementation may improve muscle strength and reduce falls [29]. In addition to its musculoskeletal effects, vitamin D plays a protective role against cardiovascular disease, which often adds to the morbidity and mortality of lupus. The Framingham Offspring Study found that 25(OH)D levels less than 15 ng/ml increase the risk of a first cardiovascular event by 62% in hypertensive patients [30]. Vitamin D may also play an important role in preventing other common complications of lupus, such as cognitive dysfunction, metabolic syndrome, and infection [31].

Moreover, lower 25(OH)D levels were encountered among our patients as disease activity increased, which was confirmed by another study [32]. They concluded that reduced levels of vitamin D in SLE patients occurred particularly in those patients with high disease activity scores and ANA positivity, suggesting that vitamin D-dependent B cell regulation may play an important role in maintaining normal B cell homeostasis, and that decreased levels of vitamin D may contribute to B cell hyperactivity in SLE patients. Borba et al. [33] found that levels of 25(OH)D were negatively correlated with SLEDAI. They explained that by the fact that vitamin D has been suggested to modulate immunological pathways and could contribute to SLE development, activity and progression, and thereby may play a role in pathogenesis and treatment of SLE. On the other hand, other authors did not find a significant correlation between 25(OH)D level and SLEDAI score [15,21,25,34]

Therapy given to our SLE patients had a significant effect on their 25(OH)D levels where significantly lower 25(OH)D levels were encountered as the dose and duration of prednisone increased. The latter data confirms that the relationship of vitamin D levels to dose and duration of prednisolone also reflects disease activity since SLE patients on larger prednisolone doses and for longer durations are the more severe cases with higher SLEDAI scores. Toloza et al. [34] found that cumulative corticosteroid exposure in SLE patients was associated with low vitamin D levels, while Chen et al. [25] found no correlation between vitamin D level and steroid use in SLE. David et al. [35] found that long-term therapy with high dose oral corticosteroids (prednisone >1 mg/kg/day or equivalent) often results in bone loss and corticosteroid-induced osteoporosis which predominantly affects trabecular bone, and thus steroid therapy should be decreased to the minimal effective dose. The mechanisms by which corticosteroids cause osteoporosis include its antivitamin D action by reducing the absorption of calcium from the gastrointestinal tract and increasing its renal excretion. This results in a net deficit of calcium, which if not corrected will result in secondary hyperparathyroidism with a consequent increase in bone resorption. In addition, corticosteroids cause reduced bone formation due to their effect on osteoblast activity, resulting in decreased matrix synthesis and a decreased active life span of osteoblasts [36].

On the other hand, higher 25(OH)D levels were encountered as frequency of use of HCQ increased, which was

confirmed by another study [14]. The role of HCQ in vitamin D metabolism is somewhat complex. Anti-malarials inhibit the 1α-hydroxylation of 25(OH)D, thus decreasing the levels of the most active form of vitamin D [37]. Huisman et al. [19] found lower $1,25(OH)_2D_3$ levels in patients with lupus treated with HCQ, although circulating 25(OH)D levels did not differ between treated and untreated patients. Therefore, it could be argued that anti-malarials spuriously increase 25(OH)D levels, at the expense of reducing the metabolically active form, 1, $25(OH)_2D_3$ [19].

In the current study, we examined the relation between 25(OH)D and each of the clinical and laboratory risk factors. From those factors, SLEDAI score, photosensitivity and photoprotection were significant predictors of vitamin D level. Similar to our results, Ruiz-Irastorza et al. [15] and Kamen et al. [16] found that photosensitivity and photoprotection were significant predictors of vitamin D deficiency in SLE patients, while disease duration was not a predictive variable of vitamin D deficiency.

Conclusions

In conclusion, low vitamin D status is prevalent in Egyptian SLE patients in spite of plenty sun throughout the year, and its level is negatively correlated to disease activity. Future studies looking at a potential role of vitamin D in the pathophysiology and treatment of SLE are warranted and further intervention studies are required to confirm a direct relationship between vitamin D status and SLE disease activity.

Conflicts of interest

Authors declare that there was no financial support and there are no conflicts of interest in the submitted manuscript.

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