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A 5-Year Monitoring SLE and Vitamin-D-Supplementation Reveals Significant Reversal in Most SLEDAI Scores and the Need for Objective Testing in Primarily Young Obese Women

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Abstract: Systemic lupus erythematous (SLE) is a devastating autoimmune disease with a sequalae of clinical manifestations. The inverse relationship between vitamin-D-deficiency (VDD) and SLE is vague. We examined relationships, tests markers, and the consequences of vitamin-D-(VD)-supplementation on SLE over a-5-year (2018-2023)-follow-up using retrospective case-controlled design. Data collected at 2-time-points between 5th and 15th months after the 1st-visit and disease activity measured using SLEDAI-2K score and laboratory records. Out of 1,207 patients, we selected 23 complying criteria. Results indicated (39%, n=9) were always positive for the anti-dsDNA and responded to supplementation that lowered SLEDAI score from 10 to 6.8 while equal number of patients (39%, n=9) were also always negative and did not respond. Nevertheless, 13% (n=3) and 9% (n=2) were positive in the 1st and 2nd visits, and while scores increased on both visits, VD doubled in the former and reduced by half in the latter. The antinuclear antibody was inconsistent with 56.5% only were always positive with SLEDAI scores remaining at 5 in both visits with minimal increase in VD (6ng/ml). The clinically consistent signs in both tests, makes it plausible that proteinurea, blood components, or genetically deficient complement interfered in negative cases. Particularly, the rates of platelets, WBC, ESR and the declining CPR with low serum complement levels strongly implied lupus glomerulonephritis for complement activation within the kidney and ceased in serum. These are manifested by arthritis, Hematurea, proteinurea, pyurea and are remarkably consistent with selective activities in the primarily young, obese females. The findings have significant implications on age-, gender-, and obesity-specific diagnosis and management. Future large-scale studies are warranted for the young target population with sunlight exposure and stable genetics. The major limitation of the study was the sample size due to extreme inclusion criteria and short intervention.

Keywords: SLE; Vitamin D supplement; Vitamin D deficiency in SLE; autoimmune disorder

1. Introduction

Systemic lupus erythematous (SLE) is a devastating multisystemic autoimmune inflammatory disease with diverse clinical manifestations(1). This includes but not limited to constitutional symptoms, rashes, arthritis, hematologic abnormalities, and nephritic complications. It predominantly affects woman in their childbearing age, exhibiting a dramatic gender-based differences in female to male ratio of 13:1 (1). While the exact cause of SLE remains unclear, factors such as immunological, hormonal, and genetic predisposition are considered likely contributors (2).

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Despite the worrying progression and complexity of SLE, there is a severe paucity in high quality data in a staggering rate of 80% of global countries with significant variations geographically and paradoxical high prevalence in developed countries (3 Tian et al., 2022).

The diagnosis of SLE relies on a combination of clinical manifestations and serological tests, with well-established criteria evolving over time. The 1989 American College of Rheumatology classification criteria and the revised 1997 version, along with the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria, serve as reliable benchmarks, exhibiting high sensitivity and specificity (4, 5, 6). Many tools have proven effective in monitoring SLE disease activity. One being the SLE Disease Activity Index 2000 (SLEDAI-2K), which is the most used worldwide(7). The strategies of SLE management aim to mitigate disease activity, prevent organ damage, manage symptoms, and minimize flare triggers through patient education, active monitoring, and interventions ranging from immunomodulators to biologics if necessary (Fava & Petri, 2019)

Vitamin D is a steroid hormone that has a well-known role in calcium homeostasis and bone metabolism. The main source of Vitamin D is the radiation of 7-dehydrocholestrol by ultraviolet B through the skin, then hydrolyzed further to 25-hydroxyvitamin D in the liver which is the major circulating form of vitamin D in serum. Further hydrolyzation occur in kidneys which converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (8). The Vitamin D receptor (VDR), present in numerous cell types, including immune cells like antigen-presenting-cells, T and B lymphocytes, underscores the role of Vitamin D in immune response modulation (9). An inverse relationship has been established between Vitamin D level and SLE disease activity (9). Thus, Vitamin D deficiency (VDD) and insufficiency are common in patients with SLE, as they are advised to avoid sunlight due to photosensitivity (10) as well as chronic use of medications and renal insufficiency may alter vitamin D metabolism, therefore increasing the risk (11).

The Vitamin D deficiency exhibits significant demographic variations in different geographic countries causing differences in disease activity and damage in SLE and leading to interest in studying the relationship between the two. Several studies have investigated the prevalence of VDD in patients with SLE, shedding light on its impact on disease activity. For instance, Kamen et al. (2006) observed 151 SLE patients in the southeastern US, revealing that 62% exhibited vitamin D deficiency, defined as serum 25-hydroxyvitamin D (25(OH)D) levels below 30 ng/mL, with a mean level of 20.1 ng/mL (SD=7.2), and only 16% (in USA) had adequate vitamin D levels (≥30 ng/mL) (12). Similarly, Toloza et al. (2010) conducted a cross-sectional study in 229 women with SLE from 14 Latin American countries and reported a prevalence of vitamin D insufficiency at about 45% (Latin America). Vitamin D insufficiency was defined as serum 25(OH)D levels between 10 and 30 ng/mL. The mean level of 25(OH)D was 25.8 ng/mL (SD=10.3), and only 24% of Chinese patients had adequate levels of vitamin D (≥30 ng/mL) (13). Furthermore, Ruiz-Irastorza et al. (2008) included 96 SLE patients from Spain and found that 51 (53%) had VDD, defined as serum 25(OH)D levels less than 20 ng/mL. The mean level of 25(OH)D was 19.1 ng/mL (SD=7.4), and only 4 patients (4%) had adequate levels of vitamin D (≥30 ng/mL)(10). In Saudi Arabia, Damanhouri et al. investigated the prevalence of vitamin D inadequacy in 165 SLE patients compared to 214 control group of non-SLE volunteers and measured serum 25-hydroxyvitamin D levels. Vitamin D sufficiency was defined as a serum 25(OH)D level >75 nmol/L (>30 ng/ ml). The study found that the prevalence of vitamin D inadequacy and deficiency was significantly higher in SLE patients compared to the control group, with p values < 0.0001. Only 1.2% of patients with SLE had adequate levels of 25(OH)D compared to 45% of the control group, which had a p value < 0.0001. Mean serum levels of 25(OH)D in SLE patients with vitamin D inadequacy and deficiency compared to the control group showed p values < 0.0001 and 0.0152, respectively (14).

Several studies have explored the relationship between VDD and disease activity in SLE. However, a great deal of variations remain in the levels, mechanisms, and assessment criteria in different countries. One study in Hong Kong found an independent association between VDD and higher disease damage scores in SLE patients. Another study in Egypt observed a prevalent VDD in 64% of SLE patients, with a significant negative correlation between vitamin D levels and disease activity. The odds ratio for disease activity in patients with VDD was 2.52 (15, 16). It is not clear what

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mechanism is involved in this relationship. There was a pattern of calcidiol deficiency with high calcitriol serum levels and a high vitamin D hydroxylation efficiency ratio associated with risk of SLE in Mexican patients (17). In addition to calcidiol deficiency, inadequate vitamin D dietary intake, and non-adherence to a DP rich in vitamin D food sources was identified as a cause for high cardiometabolic risk in SLE cases in Mexico (18). In Pakistan, obesity was a potential risk factor for vitamin D and testosterone deficiency (19).

Vitamin D supplementation has been a useful trial in the management of SLE. A controlled trial supplementing vitamin D was focused on adolescents and young adults with juvenile SLE. After a six months' time course of supplementation, the group receiving vitamin D had higher 25(OH)D levels compared to the placebo group. Additionally, 70% of the vitamin D group achieved 25(OH)D levels ≥30 ng/ml, while none in the placebo group reached this level. Fatigue scores improved significantly in the vitamin D group, particularly in social life and during exercise and medium efforts. In conclusion, vitamin D supplementation may alleviate fatigue in adolescents and young adults with juvenile SLE (20).

It is noteworthy that SLE is a complex autoimmune disease, with a heterogeneous clinical presentation and course where several factors can aggravate the outcome of the disease. The VDD is one of the potential risk factors for SLE; however, several other factors exist that complicate the disease phenotype. For instance, genetic predisposition, environmental triggers, hormonal imbalances, and infections all contribute the complexity of the outcome. Nevertheless, VDD is the predominant factor vaguely associated to SLE patients, with prevalence rates ranging from 45% to 62% in different studies. Vitamin D levels are inversely associated with disease severity and damage. Although vitamin D supplementation reduces disease activity in SLE patients, more research is needed to fully understand the frequency, role, optimum dose, and patient demographics involved. Thus, there is a significant global variation in results prompting the need for more investigations. For instance, at 30 ng/mL, with a mean 20.1 ng/mL (SD=7.2), only 16% in USA had adequate levels while VDD was 45% in Latin America at normal levels between 10 and 30 ng/mL, and the mean of 25.8 ng/mL (SD=10.3). Only 24% of Chinese patients had adequate levels of vitamin D (≥30 ng/mL) (12). In Spain, 53% had VDD at levels less than 20 ng/mL and mean of 19.1 ng/mL (SD=7.4), and only 4 patients (4%) had adequate levels of vitamin D (≥30 ng/mL)(9). However, in Saudi Arabia, at VDD set at (>30 ng/ ml) only 1.2% of patients with SLE had adequate levels. For these geographic diversities among mosaic genetic population structures globally, it was imperative to attempt to establish a threshold specific to this region that is unique in having fascinating genetic stability, nutrition, culture, and conserved lifestyle. The aim of this study was to identify and evaluate the association between VDD and SLE disease activity to improve patient adherence to doses and for disease management strategies specific to the region.

2. Materials and Methods

This study was a retrospective case-controlled using records of demographic data, clinical manifestations, and laboratory results of patients diagnosed with SLE extracted from the medical records of the rheumatology clinic at King Salman Hospital in the Hail Region, Saudi Arabia. The study period spanned from January 2018 to August 2023, with a focus on individuals aged 18 years and older who met the stringent 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE. Therefore, the study subjects comprised individuals diagnosed with SLE attending the rheumatology clinic at King Salman Specialist Hospital. Inclusion criteria were patients meeting the 2012 SLICC criteria for SLE and aged 18 years or older, while exclusion criteria included individuals with malabsorption, renal and liver diseases, inflammatory bowel disease, and those on antifungal or anticonvulsant medications. This research adopted a retrospective cohort study design, and the sample size is contingent upon the data available from the center.

A review of 1,207 patient records from the rheumatology clinic at King Salman Specialist Hospital was conducted. After removing duplicates and excluding patients with interfering conditions other than SLE, 92 potential SLE cases remained. Further application of

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inclusion/exclusion criteria and data availability resulted in a final sample of 23 patients for analysis. Patients were recorded on two occasions based on the avaliabilty of data and SLEDAI parameters, we excluded periods less than 3 months. The study also sought to assess patients' adherence to vitamin D supplements by examining serum 25-hydroxyvitamin D levels in comparison with available SLEDAI scores on two occasions. The duration of these two occasions varied from 5 months (155 days) to 15 months (470 days), while the mean duration from 1st to 2nd visit was 299±84.16 days. Disease activity and vitamin D levels were measured on two occasions, each separated by at least 5 months, with the specific choice of occasions guided by the availability of the SLEDAI-2K score variable. The investigation aimed to discern the impact of vitamin D levels on disease severity in patients with normal and deficient vitamin D levels, comparing the two groups and reevaluating them after vitamin D supplementation.

Written informed consent for participation in research activities was obtained from all patients during hospital visits or admissions. Ethical approval (IRB) was granted and (Approved) by the Hail University Ethical Committee and written ethical consent was obtained from all participating individuals. Data were meticulously collected from King Salman Hospital, with stringent application of predefined inclusion and exclusion criteria to ensure the methodological rigor and reliability of the study sample.

The laboratory assessments, conducted at approximately three-month intervals between visits, included a comprehensive panel encompassing full blood count, complements (C3 and C4), Anti ds-DNA titer, renal function tests, ESR, CRP, serum calcium, and serum 25-hydroxyvitamin D. Vitamin D deficiency and insufficiency were defined as serum 25-hydroxyvitamin D levels below 20 ng/mL and between 21-29 ng/mL, respectively. Recorded information also included the use of steroids, immunosuppressive drugs, and Vitamin D supplementation. The therapeutic administration of Vitamin D involved an initial dose of 50000 IU/week for 8-12 weeks, followed by a maintenance dose of either 50000 IU/month or 10000 IU/week (21). The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) served as the primary tool for defining disease activity and clinical remission, where a score of ≥3 indicated moderate to high disease activity, while a score of <3 suggested low disease activity (22).

Statistical Analysis:

Relevant data was extracted from medical records, reviewed, and normalized. The statistical analysis was conducted using SPSS version 26, focusing on key parameters related to Disease Activity and severity in SLE patients. The primary measure for assessing disease severity was the SLEDAI-2k scoring system. Additionally, other markers such as ESR, CRP, and complement levels were considered in evaluating disease severity. Demographic and clinical characteristics were thoroughly examined. Age and Body Mass Index (BMI) were measured and reported as mean ± standard deviation (SD), providing insight into the overall population distribution. The gender distribution was analyzed and presented in terms of frequency and percentages, offering a comprehensive view of the sample composition. Continuous variables such as complete blood count (CBC), CRP, urinalysis, and total SLEDAI scores at the 1st and 2nd visits were presented as mean ± SD. Categorical variables, including the usage of corticosteroids, immunosuppressants, antimalarial medication, and the supplementation of vitamin D and calcium, were reported in terms of frequency and percentages.

The SLEDAI subcategories were meticulously analyzed, with their frequencies and percentages documented. The total SLEDAI score was presented as mean ± SD, providing a holistic perspective on disease severity. To explore the relationship between vitamin D levels and Disease Activity in SLE patients at the 1st and 2nd visits, a Pearson correlation coefficient was employed. The comparisons between the 1st and 2nd visits were conducted using paired t-tests. These tests were instrumental in assessing changes in ESR, CRP, complement levels, and total SLEDAI scores over time. A significance level of $p < 0.05$ was set to identify statistically meaningful differences between the two visits.

3. Results

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A record of 23 patients with SLE patients attended King Salman Hospital between 2018 and 2023 were included in the study. The mean age of the patients was 38.00±9.34. The mean BMI was 32.07±8.05, showing that most patients were falling into the obese range of measure of body fat categories. The distribution of cases according to gender has shown that 21 (91.3%) patients were females, and 22 (95.7%) patients i.e all except one, were Saudi nationals as shown in Table 1

Table 1. Profiles of SLE patient demographic characteristics in Ha'il, Saudi Arabia.

Variables	**Range N=23**	**Mean ± S.D.**
Age	22.00 to 60.00	38.00 ± 9.34
BMI (kg/m²)	18.29 to 49.69	32.0713±8.05644
	Frequency	**Percentage**
Gender		
Male	2	8.7
Female	21	91.3
Nationality		
Saudi	22	95.7
Non- Saudi	1	4.3

Laboratory findings of hematology and other tests as shown in Table 2a were measured in the patients' samples with reference to normal laboratory values. Among these, notably, 25-Hydroxyvitamin D levels exhibited a mean of 32.31 ng/mL. Regarding the frequency and percentage distribution of laboratory parameters, antinuclear antibody (ANA) positivity was observed in various patterns among patients tested. In addition, anti-double-stranded DNA (Anti-dsDNA) positivity was present in 43.5% of cases examined. Urinalysis revealed elevated red and white blood cell counts (WBC), as well as the presence of casts and proteins in urine samples reflecting the renal and systemic function profiles. Vitamin D supplement usage was prevalent, with 52.2% on a maintenance dose and 39.1% on a therapeutic dose. Medication usage, including glucocorticoids, immunosuppressants, antimalarials, and calcium supplementation, varied among the patients and shown in Table 2a.

Results of the second visit delineated key clinical parameters. The mean temperature was 36.9391°C with a narrow standard deviation of 0.13731°C (Del: indicating minimal variability). Furthermore, hemoglobin levels averaged at 12.5565 g/dl, WBC count was 5.8709 X10^3/uL, platelet count measured 249.0000 X10^3/uL, and Red Blood Cell (RBC) count was 4.4883 X10^6/uL. However, the serum 25-Hydroxyvitamin D levels showed variability in vitamin D status by measuring a mean of 34.9665 ng/mL, with a noticeable standard deviation of 15.65016. Calcium levels remained within the normal range, with a mean of 2.2022 mmol/L. Elevated values were observed for ESR and C3 levels, with means of 56.0435 mm/hour and 0.7903 g/L, respectively. Conversely, CRP levels exhibited a relatively low mean of 0.5616 mg/d. The mean Total SLEDAI score at the second visit was 5.82, with a standard deviation of 3.08, indicating variability in disease activity. In terms of categorical variables, ANA positivity was observed in various patterns, with the majority exhibiting negative or speckled patterns. Anti-double-stranded DNA positivity was present in 47.8% of cases. Urinalysis revealed varying levels of red and WBC, with notable proteinuria observed in 21.7% of cases and shown in Table 2b.

Table 2. a: Laboratory findings and total SLEDAI scores at the 1st -Visit in SLE patients, Ha'il, Saudi Arabia. b: Laboratory findings and total SLEDAI scores at the 2nd-Visit in SLE patients, Ha'il, Saudi Arabia.

a

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Descriptive Statistics: (*n*=23)		
	Mean	**Std. Devi**
Temperature	36.85	0.26
Hb (g/dl)	12.57	1.71
WBCs (X10^3/uL)	5.45	2.45
Platelets (X10^3/uL)	237.60	85.97
RBCs (X10^6/uL)	4.57	0.57
25- Hydroxyvitamin D ng/mL	32.31	12.42
Calcium (mmol/L)	2.18	0.21
ESR (mm/hour)	52.56	25.25
CRP (mg/d)	1.27	2.17
C3 (g/L)	0.7809	0.27890
C4 (g/L)	0.2018	0.21390
Creatinine (ummol/L)	64.7452	30.19384
eGFR	106.8696	22.57775
Proteinuria mg/day (In 24-hour)	547.6652	692.56036
Total SLEDAI score	6.78	6.76
	Frequency	**Percentage**
ANA Positive Speckled Coarse large Speckled Homogenous and cytoplasmic Homogenous and nuclear Negative N/A	11 3 3 1 4 1	47.8% 13% 13% 4.3% 17.4% 4.3%
Anti-dsDNA Positive Negative Borderline	10 11 2	43.5% 47.8% 8.7%
Urinalysis		
RBCs Nil <5 >5	13 5 5	56.5% 21.7% 21.7%
WBSs (Pyuria) Nil <5 >5 Pyuria	10 9 2 2	.43.5% 39.1% 8.7% 8.7%
Casts Nil +++	21 1 1	91.3% 4.3% 4.3%
Protein Nil + +++	16 5 2	69.6% 21.7% 8.7%
b		
Descriptive Statistics. (*n*=23)1		
	Mean	**Std. Devi**

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Temperature	36.9391	.13731
Hb (g/dl)	12.5565	2.10450
WBCs (X10^3/uL)	5.8709	2.86708
Platelets (X10^3/uL)	249.0000	91.41464
RBCs (X10^6/uL)	4.4883	.68103
25- Hydroxyvitamin D (Normal range: 30-100 ng/mL)	34.9665	15.65016
Calcium (mmol/L)	2.2022	.11591
ESR (Normal range: 0-20 mm/hour)	56.0435	27.65120
CRP (Normal range: 0-0.08 mg/d)	.5616	.45680
C3 (Normal range: 0.79-1.52 g/L)	.7903	.34300
C4 (Normal range 0.16-0.38 g/L)	.1946	.21927
Creatinine (Normal range: 49-90 ummol/L)	62.9235	30.64493
eGFR	109.3043	23.53032
Total SLEDAI score	5.82	3.08
	Frequency	**Percentage**
ANA Positive Speckled	9	39.1%
Coarse large Speckled	1	4.3%
Homogenousand cytoplasmic	4	17.4%
Negative	2	8.7%
N/A	7	30.4%
Anti-dsDNA Positive	11	47.8%
Negative	12	52.2%
Urinalysis		
RBCs Nil	13	56.5%
<5	9	39.1%
>5	1	4.3%
WBSs (Pyuria)		
Nil	11	47.8%
<5	11	47.8%
>5	1	4.3%
Casts Nil	23	100%
+	0	0%
+++	0	0%
Protein Nil	15	65.2%
+	1	4.3%
+++	2	8.7%
Positive	5	21.7

Profiles of SLEDAI subcategory scores at 1st visit were examined as shown in Table 3a. Results indicated that most of the patients did not exhibit seizures, psychosis, organic brain syndrome, lupus headache, cerebrovascular accidents, or manifestations such as alopecia, mucosal ulcers, and pericarditis. However, visual disturbances and cranial nerve disorders were present in 4.3% of patients examined. Joint-related symptoms, including arthritis, were observed in 17.4% of cases. Renal involvement, indicated by the presence of hematuria, proteinuria, and urinary casts, was noted

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in varying proportions. Additionally, cutaneous manifestations such as rash were evident in 8.7% of individuals. Immunological abnormalities, such as low complement levels and increased DNA binding, were observed in a significant proportion of patient samples at 65.2% and 43.5%, respectively. Thrombocytopenia and leukopenia were present in 8.7% of cases.

The 2nd- SLEDAI test for the 23 patients revealed that none of the patients exhibited seizures, psychosis, organic brain syndrome, lupus headache, cerebrovascular accidents (CVA), vasculitis, myositis, urinary casts, pleurisy, pericarditis, alopecia, fever, thrombocytopenia, or leukopenia at the second visit. However, arthritis was present in 21.7% of cases. Notably, a substantial proportion of patients presented with proteinuria (30.4%), while low complement levels were observed in 69.6% of cases. Increased DNA binding was noted in 47.8% of patients. Visual disturbances, cranial nerve disorders, and mucosal ulcers were infrequently reported, each occurring in less than 5% of cases and is shown in Table 3b.

Vitamin D supplement maintenance and therapeutic doses, 52%, (*n*=12), and 39%, (*n*=9), respectively, were administered. In addition, Glucocorticoid and immunosuppressive each 78.3% (*n* =18), and antimalarial drug and calcium supplementation each 82% (*n*=19) were administered between the two visits with no change. The Pearson correlation coefficient between 25-Hydroxyvitamin D levels and the total SLEDAI score at the first visit revealed a weak negative correlation of -.176 though it was not statistically significant (p = .423). Similarly, Pearson correlation coefficient demonstrated a moderate positive correlation of .311 between 25-Hydroxyvitamin D levels and the total SLEDAI score at the second visit, even though it did not reach statistical significance (p = .149).

Table 3. a: A 5-year comprehensive clinical disease profiles at 1st visit of patients in Ha'il, Saudi Arabia (2018-2023). Table3b:A 5-year comprehensive clinical disease profiles at 2nd visit of patients in Ha'il, Saudi Arabia (2018-2023).

a		
Descriptive Statistics. (*n*=23)		
	Frequency	**Percentage**
Seizure No	23	100%
Yes	0	0%
Psychosis No	23	100%
Yes	0	0%
Organic Brain Syndrome No	23	100%
Yes	0	0%
Visual No	22	95.7%
Disturbance Yes	1	4.3%
Cranial Nerve No	22	95.7%
Disorder Yes	1	4.3%
Lupus No	23	100%
Headache Yes	0	0%
CVA No	23	100%
Yes	0	0%
Vasculitis No	22	95.7%
Yes	1	4.3%
Arthritis No	19	82.6%
Yes	4	17.4%.
Myositis No	23	100%
Yes	0	0%
Urinary No	22	95.7%
Casts Yes	1	4.3%
Hematuria No	17	73.9%
Yes	6	26.1%
Proteinuria No	18	78.3%
Yes	5	21.7%

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Pyuria No Yes	21 2	91.3% 8.7%
Rash No Yes	21 2	91.3% 8.7%
Alopecia No Yes	23 0	100% 0%
Mucosal No ulcers Yes	23 0	100% 0%
Pleurisy No Yes	22 1	95.7% 4.3%
Pericarditis No Yes	23 0	100% 0%
Low complement No Yes	8 15	34.8% 65.2%
Increased DNA binding No Yes	13 10	56.5% 43.5%
Fever No Yes	23 0	100% 0%
Thromboc No ytopenia Yes	21 2	91.3% 8.7%
Leukopenia No Yes	21 2	91.3% 8.7%
b		
Descriptive Statistics (*n*=23)		
	Frequency	**Percentage**
Seizure No Yes	22 1	95.7% 4.3%
Psychosis No Yes	23 0	100% 0%
Organic No Brain Yes Syndrome	23 0	100% 0%
Visual No Disturbance Yes	22 1	95.7% 4.3%
Cranial No Nerve Yes disorder	23 0	100% 0%
Lupus No headache Yes	23 0	100% 0%
CVA No Yes	23 0	100% 0%
Vasculitis No Yes	23 0	100% 0%
Arthritis No Yes	18 5	78.3% 21.7%.
Myositis No Yes	23 0	100% 0%
Urinary No Casts Yes	23 0	100% 0%
Haematuria No Yes	22 1	95.7% 4.3%
Proteinuria No Yes	16 7	69.6% 30.4%
Pyuria No Yes	22 1	95.7% 4.3%
Rash No Yes	22 1	95.7% 4.3%

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Alopecia No Yes	23 0	100% 0%
Mucosal No ulcers Yes	21 2	91.3% 8.7%
Pleurisy No Yes	23 0	100% 0%
Pericarditis No Yes	23 0	100% 0%
Low complement No Yes	 7 16	 30.4% 69.6%
Increased DNA binding No Yes	12 11	52.2% 47.8%
Fever No Yes	23 0	100% 0%
Thrombo No cytopenia Yes	23 0	100% 0%
Leukopenia No Yes	21 2	91.3% 8.7%

2nd-Visit in SLE patients, Ha'il, Saudi Arabia.

In this study, the major clinical parameters that potentially played a role in this relationship between the first and second visits are presented in Table 4. For ESR was increased from 52.57 mm/hour at the first visit to 56.04 mm/hour at the second visit. However, CRP levels decreased from 1.28 mg/dL at the first visit to 0.56 mg/dL at the second visit. On the other hand, complement (C3 and C4) levels revealed changes even though they were minimal. However, vitamin D levels increased from a mean of 32.31 ng/mL at the first visit to 34.96 ng/mL at the second visit. The corresponding SLEDAI scores reflected a decrease in activity from a mean of 6.78 at the first visit to 5.83 at the second visit. Overall, there were changes in ESR, CRP, complement levels, Vitamin D, or total SLEDAI scores between the first and second visits in the target sample.

Table 4. Comparison of ESR, CRP, complement level, and SLEDAI scores between 1st and 2nd visits in SLE patients, Ha'il, Saudi Arabia.

Variables	Mean	Std. Deviation
ESR mm/hour at First Visit	52.5652	25.25835
ESR mm/hour at Second Visit	56.0435	27.65120
CPR mg/dL at First Visit	1.2763	2.17469
CPR mg/dL at Second Visit	0.5616	.45680
C3 g/L at First Visit	.7809	.27890
C3 g/L at Second Visit	.7903	.34300
C4 g/L at First Visit	.2018	.21390
C4 g/L at Second Visit	.1946	.21927
25- Hydroxyvitamin D ng/mL at First Visit	32.31	12.42
25- Hydroxyvitamin D ng/mL at Second Visit	34.96	15.65

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Total SLEDAI score at 1st visit	6.7826	6.76190
Total SLEDAI score at 2nd visit	5.8261	3.08445

As shown in Table 5a, the Anti-DNA test in those which were always positive 9 (39%), an increase in serum vitamin D level from 33 to 42 ng/mL was accompanied by a significant reduction in score from 10 to 6.88 between the 1st and 2nd visits, respectively. However, in those recorded positive in 1st visits only (3,13.04%), with a doubling increase in serum vitamin D levels from 19.58 to 40.43 ng/mL a concomitant paradoxical increase occurred in score from 6.33 and 7.33, between the 1st and 2nd visits, respectively. Nevertheless, in patients with positive anti-DNA in 2nd visit only (2, 8.69%), a decrease in vitamin D from 42.42 to 22.6 and increase in score from 2 to 4.5 occurred, respectively. Patients with "always negative Anti-DNA test (9, 39.13%) had a slight decrease in score (4.77 to 4.5) and nearly stable vitamin D levels (33.50 to 28.52 ng/mL) in both visits.

Table 5. a. Patient groups based on Anti-dsDNA antibody, vitamin D supplementation, and associated SLEDAI score. b. Patient groups based on antinuclear antibody, vitamin D supplementation, and associated SLEDAI score.

a					
Anti-DNA	N=23	Mean SLEDAI score at 1st vistit	Mean SLEDAI score at 2nd visit	Mean 25-hydroxyvitamin D ng/mL at 1st visit	Mean 25-hydroxyvitamin D ng/mL at 2nd visit
Always Positive	9 (39.13%)	10 (Moderate to Severe)	6.88 (Moderate to Severe)	33.13	42.33
1st Visit +ve and 2nd -ve	3 (13.04%)	6.33 (Moderate to Severe)	7.33 (Moderate to Severe)	19.58	40.43
1st Visit -ve and 2nd +ve	2 (8.69%)	2 (Mild)	4.5 (Moderate to Severe)	42.42	22.6
Always negative	9 (39.13%)	4.77 (Moderate to Severe)	4.5 (Moderate to Severe)	33.50	28.52
b					
ANA	N=23	Mean SLEDAI score At 1st visit	Mean SLEDAI score at 2nd visit	Mean 25-hydroxyvitamin D ng/mL at 1st visit	Mean 25-hydroxyvitamin D ng/mL at 2nd visit
Always Positive	13 (56.52%)	5.30 (Moderate to Severe)	5.15 (Moderate to Severe)	28.11	34.47
1st Visit +ve and 2nd -ve	0 (0.00%)	NA	NA	NA	NA
1st Visit -ve and 2nd +ve	1 (4.34%)	6 (Moderate to Severe)	6 (Moderate to Severe)	40	50
Always negative	2 (8.69%)	5 (Moderate to Severe)	6 (Moderate to Severe)	31.76	40.5

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Recorded on one visit only as +ve	5 (21.73%)	9 (Moderate to Severe)	7 (Moderate to Severe)	37.87	29.2
Recorded on one visit only as -ve	1 (4.34%)	14 (Moderate to Severe)	6 (Moderate to Severe)	26.45	20
Never recorded (recorded elsewhere)	1 (4.34%)	12 (Moderate to Severe)	8 (Moderate to Severe)	58.47	59

The results of the ANA marker (Table 5b) were general and not conclusive and not so consistent to draw any conclusion, as shown below. For instance, only 56.5% were always positive with SLEDAI scores remaining at 5 in both visits with minimal increase in vitamin D (6ng/ml). Nevertheless, the puzzling inversion results were those which were either always negative (9%) with SLEDAI scores increasing from 5 to 6 in spite of significant increase in vitamin D from ~32 to 40.5 ng/ml. Those positive only on second visit (4.3% one patient) with SLEDAI score remained at 6 in both visits albeit serum vitamin D increased from 40 to 50 ng/ml. However, those recorded in one visit only as positive (5, 21.73%) showed reduction in score from 9 and 7 and in serum vitamin D levels as well from ~ 38 to 29 ng/mL. Another inversion was only one patient recorded in one visit only as negative (4.34%) with a score of 14 that reduced to 6 with the reduction in serum vitamin D from 26.45 to 20 ng/mL.

Discussion

There has been an ongoing devastating struggle to establish a convincing association in the role of VDD on the SLE aggravation. This is particularly significant in women and public health in the region owing to the endemic spread of VDD, SLE, epigenetic, diabetes, obesity, and genetic predispositions. This study was a 5-year comprehensive profiling for the investigation of laboratory, clinical, demographic, and syndromic findings at 2-visit intervals to understand any tangible impact of VDD in aggravating SLE in Saudi patients. The analysis of demographic and clinical characteristics of the study population, including laboratory values and disease markers, revealed significant association in about 50% of patients consistent with widely known inverse relationship of VD and SLE. In addition, the gender-based prevalence of SLE in women of reproductive age in this study is consistent with other reports (1). However, although late-onset SLE is commonly known to occur after the age of 50 (23, 24) the selective predominance in young otherwise mostly healthy women in this study is worth further studies. However, in this study, nearly a similar percentage of patients showed negative anti-dsDNA test results. This test behavior is a common phenomenon occurring in nephrotic-range patients with heavy proteinuria causing negative serologic findings due to loss of antibodies in the urine, tissue deposition, or other factors relevant to our results (25, 26). One of the major pitfalls in several studies is the screening procedures alerting for an objective testing and reliance on clinical syndromes of SLE. As such, the slowly climbing rates of platelets, WBC, ESR and the declining CPR with low serum complement levels implied potential nephritis for associated lupus glomerulonephritis due to infection (27, 28, 29). These are further manifested in arthritis, Hematurea, proteinurea, pyurea, where low serum complement levels in patients with glomerulonephritis most often result from activation of complement within the kidney (30, 31). Although patients with genetic or congenital complement deficiencies are also more prone to develop glomerulonephritis with local complement activation (32)

Despite significant differences in population genetic structures, this latter findings were consistent with a USA study where the clinical importance of a minor increase in VDD was relatively modest with no evidence of additional benefit beyond a level of 40 ng/mL(33). This was also in agreement with demographic profile of predominantly females in both studies albeit Saudi patients were younger at a mean age of 38.00±9.34. The latter results were also aligned with the typical epidemiology of SLE in the country (34). However, the patients exhibited a high prevalence of obesity, reflecting the well-established association between SLE and obesity (35).

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The study population exhibited a high prevalence of VDD, with a mean 25-hydroxyvitamin D level of 32.31 ng/mL at the first visit. This results was consistent with existing literature indicating that VDD is common among SLE patients (36). However, despite initiating Vitamin D supplementation, the subsequent analysis at the second visit revealed a minimal increase to a mean of 34.96 ng/mL. This observation was in agreement with studies that highlighted the challenges of achieving optimal Vitamin D levels through supplementation, emphasizing the multifactorial nature of Vitamin D metabolism (37, 39). Apparently, the aforementioned study was on long-care facility patients living indoors in a sun-deprived situations with difficulty to meet their need for vitamin D, especially those with higher dietary recommendations than the rest of the population due to their age and/or medical conditions. Nevertheless, despite the younger age and a year-round sunlight exposition in this region, they had the same challenges of achieving optimal Vitamin D levels through supplementation in this study. In addition, unlike the USA counter parts in the above study, this study group was able to independently manage their daily living activities and the administration of the supplement. While a greater need for Vitamin D by senior adults is justified by chronic disease societies such as the Endocrine Society (30) due to greater risk for chronic diseases, albeit this did not fully apply to this study group. Thus, although therapeutic dosing with adequate response for the target population is the effective strategy to achieving optimal levels over dietary intakes from natural or fortified foods, questions about the practical application remain (41).

Different types of blood and other tests indicated the presence of different formats of disease activity. For instance, even though increasing at pace, the persistently lower level of platelet counts below Saudi Arabia range during both visits (normal range in Saudi Arabia 285.16–295.76) could be an indication of inflammatory or infectious marker for SLE (42). Consistent with the above study, patients developed kidney functionality issues with lower butterfly erythema in this study. The elevated levels of ESR in both visits in this study prompted flare and infection and together with immunological abnormalities, such as positive ANA and Anti-dsDNA, were consistent with the characteristic of autoimmunity in SLE. In an ESR/CRP comparative study, a correlation was found in cases of infection and flare; however, ESR was higher in flare than in infection ($p = 0.048$) (43) and ESR, serositis, and anti-dsDNA antibodies prompted disease activity irrespective of infections. On the other hand, Anti-dsDNA antibodies were most sensitive for detecting flares (74%); whereas serositis, proteinuria with erythrocyturia, anti-dsDNA antibodies, C3 reduction, and ESR values ≥ 2 were most specific (43). Nevertheless, ESR and CRP levels persisted after the Vitamin D supplementation prompting for no improvement in contrast to others (38). This study reported persistent renal involvement, as evidenced by proteinuria, hematuria, and urinary casts, (42). Renal manifestations were common in SLE and are associated with increased disease activity and severity; however, the absence of significant improvements post Vitamin D supplementation raised questions about the mechanism(s) involved or specific impact of the vitamin in the short term. A link between vitamin D deficiency and elevated CRP in SLE patients has been reported, potentially indicating a modulatory role of vitamin D in inflammation (44). In the current study, an increase in 25-hydroxyvitamin D levels by 2.65 ng/mL was followed by a decline between the first and second visits in CRP levels (by 0.7 mg/dL) and a decrease in SLE Disease Activity Index (SLEDAI) score (by 0.96) was reported. It is known that CRP mostly indicates inflammatory reactions, and this result might potentially indicate a role for VD in reducing inflammatory activity of SLE and/or infections (45). Furthermore, the decrease of proteinuria and hematuria in a few patients in this study, was probably a consequence of a potential protective effect of vitamin D (33) since lupus nephritis can manifest in a range of urinary abnormalities, including proteinuria, hematuria, and cellular casts. While the mechanisms driving lupus nephritis are complex, involving immune complex deposition and inflammation within the kidney, emerging evidence suggests a potential link between vitamin D levels and the development of severe hematuria in SLE (46). Nevertheless, thrombocytopenia was observed in two patients at the first visit but in none at the second. This and the persistently lower platelet levels during both visits could be an indication of inflammatory marker for SLE (41) for the development of kidney functionality issues with lower butterfly erythema in this study. A study

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indicated that severe vitamin D deficiency was significantly associated with thrombocytopenia in SLE patients. Lower vitamin D levels had a higher prevalence of hematological manifestations, including thrombocytopenia, suggesting a correlation between vitamin D status and platelet counts in this population (47).

The correlation analysis was aimed at exploring the relationship between Vitamin D levels and SLE disease activity. At the first visit, a weak negative correlation was observed, suggesting that higher Vitamin D levels were associated with lower disease activity. This finding is consistent with some literature suggesting an inverse relationship between Vitamin D levels and disease activity in autoimmune conditions, including SLE (9, 43). However, the lack of statistical significance in this study aligns with the inconclusive evidence in the existing literature, emphasizing the need for further investigation. At the second visit, a moderate positive correlation between Vitamin D levels and disease activity emerged. This unexpected correlation is not consistently reported in the literature, and the lack of statistical significance underscores the complexity of the relationship between Vitamin D and SLE disease activity.

The paired t-test results comparing clinical parameters between the first and second visits indicated that Vitamin D supplementation and follow up from first to second visits, even though result in a remarkable change, drew attention to levels of ESR, CRP, complement levels, and calcium. This finding implied a potential role for a concerted mechanisms of the markers in an alternative modulation of inflammatory and immune response emphasizing the need for further exploration in the context of SLE. The lack of substantial improvement in other sequalae of clinical parameters and the emergence of unexpected correlations between Vitamin D levels and disease activity underscored the need for vertical studies. Larger, longitudinal studies are warranted to elucidate the intricate relationship between Vitamin D and SLE disease progression comprehensively. Moreover, exploring the impact of Vitamin D on specific SLE manifestations, such as renal involvement, could provide valuable insights into targeted therapeutic strategies.

Comparative analysis of patient groups based on Anti-dsDNA antibody, vitamin D supplementation, and associated SLEDAI score revealed consistent results in groups of patients. The test was always positive in 39% (n=9) with a concomitant increase in serum vitamin D level from 33 to 42 ng/mL and a significant reduction in score from 10 to 6.88 between the two visits. Renal involvement was evidenced by hematuria, proteinuria, and urinary casts. In addition, skin rash, immunological abnormalities including low complement levels, thrombocytopenia, leukopenia, and kidney issues with lower butterfly erythema were observed. The elevated levels of ESR in both visits in this study prompted flare and/or infection and together with immunological abnormalities, such as positive ANA and Anti-dsDNA, were consistent with the known characteristics of autoimmunity in SLE. However, the mechanisms were not clear in those always negative, positive in 1st or 2nd visits only and that of antinuclear tests owing to the test conditions in cases of heavy protenurea and blood components masking antigens.

Conclusion

In this study, we have monitored the relationships, tests markers, the consequences of vitamin-D-(VD)-supplementation and the corresponding SLEDAI scores over a-5-year (2018-2023)-follow-up. The study provides valuable insights and the potential reasons for the nuanced relationship between Vitamin D levels and SLE disease activity in young, obese women with sunlight exposure. We provide proof of concept that VD supplementation significantly improved scores in about 50% of patients with consistent laboratory and anti-dsDNA tests. Nevertheless, it is plausible that the clinically relevant signs including heavy proteinurea, Hematurea, proteinurea, pyurea increasing platelets, WBC, ESR and the declining CPR with concomitant decrease in serum complement strongly implied its local activation in kidneys causing severe lupus glomerulonephritis with excretion of proteins and blood components that impeded anti-dsDNA and antinuclear tests. The findings have significant implications on age-, gender-, and obesity-specific diagnosis and management. Future large-scale studies are warranted for the young target population with sunlight exposure and stable

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genetics. The major limitation of the study was the sample size due to extreme inclusion criteria and short intervention.

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Abbreviations

The following abbreviations are used in this manuscript:

SLE	Systemic Lupus erythromatus
VDD	Vitamin D deficiency
VD	Vitamin D
ANA	Antinuclear antibody
Anti-dsDNA antibody	Anti-double Stranded DNA antibody

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