

Evaluation of Vitamin D Status in Sudanese Patients with Systemic Lupus Erythematosus Receiving Vitamin D and Calcium Supplements

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Abstract: **Background:** Systemic Lupus Erythematosus (SLE) is a potential fatal autoimmune disease. Vitamin D deficiency is more prevalent among SLE patients than the general population. Over the past years, many studies worldwide have been carried out to investigate the role of vitamin D in SLE patients. Therefore, this study is aimed to evaluate and summarize the evidence of the association between vitamin D level and different doses of vitamin D and calcium supplements. **Methodology:** A cross-sectional case-control study of patients with SLE who were following-up in various private clinics at Khartoum state during February-May 2017. Demographic and clinical data were recorded and vitamin D levels of patients were measured using ELISA techniques. Chi square tests, One-Way ANOVA, Independent T-tests, and Pearson tests were used for statistical analysis. A p-value <0.05 was considered significant. **Results:** A total of 120 subjects (60 cases [35 SLE patients and 25 Lupus nephritis (LN) patients] with mean age of 31±9 years) and (60 healthy subjects as control with mean age of 29±8 years) were enrolled in the study. The levels of vitamin D were found to be elevated significantly in patient with SLE compared to control group (P-value=0.002). Serum concentration of Vitamin D as total 25-hydroxyvitamin D (25(OH)D) was measured using enzyme linked immunosorbent assay (ELISA). Deficient, insufficient, sufficient, and potential toxic vitamin D levels in corresponding to serum 25(OH)D concentrations of <25, 25-72.5, 75-250, and >250 nmol/L, were found in 6.7%, 50.0%, 31.7%, and 11.7% of the patients, and 13.3%, 55.0%, 31.7%, and 0.0% in control, respectively (P-value: 0.037). The mean levels of vitamin D among subgroup patients did not show significant correlation, although it was lower in patients with SLE (n=35, 100±17.10) than in those with LN (n=25; 111.34±71; P-value: 0.345). The mean levels of 25(OH)D according to type of supplementation were highly significant when compare with patients who do not use vitamin D or calcium supplement (41.62±27.29 nmol/L), using vitamin D and calcium supplement (233.85±128.44 nmol/L; P-value:0.000), using vitamin D supplement only (176.15±139.70 nmol/L; P-value:0.006), and using calcium supplement only (80±34.11 nmol/L; P-value:0.022). The mean of 25(OH)D levels was significantly variable when the comparison is among patients who do not receive any dose of vitamin D or calcium supplement (55.36±35.00) and patients with high dose of vitamin D (50,000-60,000 IU), moderate dose (400-1000 IU), and low dose (100 IU) corresponding to means (338.50±114.86 nmol/L; P-value: 0.000), (196.00±102.05 nmol/L; P-value: 0.000) and (83.50±33.87 nmol/L; P-value: 0.002), respectively. There was no correlation between duration of disease and vitamin D levels (R=0.010; P-value=0.939). Vitamin D levels and duration of vitamin D supplementation had positively correlated (R=0.374; P-value=0.003). **Conclusion:** There is a convincing evidence to support the association of low vitamin D levels in SLE patients who do not use vitamin D or calcium supplement and significant correlation between vitamin D levels and taken supplements in different vitamin D doses and/or calcium. Continuous monitoring should be performed to avoid toxic and deficient levels.

Keywords: Vitamin D, Systemic lupus erythematosus, Vitamin D supplementation, 1,25-Dihydroxyvitamin D

1. Introduction

1, 25-Dihydroxyvitamin D (calciferol) is the most active form of vitamin D and considered as an essential steroid hormone. It has a role in bone homeostasis via calcium and phosphorus metabolism [1-4]. Vitamin D is associated with body's immune system, multiple studies have shown that vitamin D have an influence on antigen presenting cells, modulates the secretion of cytokines and involved in the regulations and proliferations of Th1 and Th17 lymphocyte [4,5].

The major source of vitamin D is the exposure of skin to sunlight (UV) and diet [6,7]. Vitamin D has two forms: ergocalciferol (vitamin D2) which found in plants and cholecalciferol (vitamin D3) mainly found in animals [7]. In the body, vitamin D undergoes hepatic metabolism to 25-hydroxyvitamin D (25(OH)D), this form is conventionally measured in serum in clinical assays of vitamin D status. In the kidney, 25(OH)D is further metabolized to 1,25-dihydroxyvitamin D by parathyroid hormone (PTH), which works by binding to the vitamin D receptor (VDR) located in the nuclei of target cells [7,8].

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that attacks healthy tissue of almost any organ system. It can affect the skin, joint, kidneys, brain, and other organs, with high presentation and course [9,10]. Symptoms and complication of the disease can vary widely, such as kidney dysfunctions, heart problems, suffering from stroke or develop lung inflammation [11]. The cause of autoimmune disease is not fully known. SLE is more common among women and have higher risk than men, especially African-American and Asian women. It may occur at any age. However, it appears most often in people between the ages of 15-44. About 15% of patients experience the onset of symptoms before age 18 [9, 12].

Vitamin D deficiency is particularly prevalent and profound in racial groups with higher levels of melanocytes (pigment-producing cells that help block UV photons) about 12% of apparently healthy African Americans have been found to have severe vitamin D deficiency [13].

The interest in the association between 25(OH) D) and SLE started developing early in the last decade when the vitamin was related to low bone mass in patients with SLE[14]. Several studies have reported that vitamin D deficiency is

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more prevalent in SLE patients than in the general population [15] and this is due to universal recommendation and medical advice to patients to avoid exposure to sunlight. Moreover, medications such as glucocorticoids and hydroxychloroquine that used in SLE management may interfere with the catabolism (enzymatic breakdown) of vitamin D, could eventually contribute to its deficiency and serum levels [13, 15].

Vitamin D deficiency has been proposed as environmental trigger of disease onset as a contributor to increased SLE activity [2].

There may be a higher vitamin D requirement for patients who are at risk for developing autoimmunity and for those who already have an autoimmune disease such as SLE [16]. Hydroxychloroquine (HCQ) has been successfully used for treatment of SLE for over 70 years and it has a good reputation for controlling the dermatological complications, reducing flare, inflammatory, thrombosis, increased longevity, improved lipid, better glycemic control and blood pressure [17,18,19]. In SLE, HCQ is preferred due to its lower incidence of gastrointestinal complication, and using HCQ during pregnancy has benefits to mother with SLE and her child because it control the disease activity which is a factor that affect pregnancy outcome [17].

2. Materials and Methods

This is cross-sectional case-control study that includes 60 patients with SLE disease who were following up at four Rheumatology clinics. It was conducted in Sudan-Khartoum state from February to May 2017.

All cases of SLE were diagnosed by a rheumatologist according to American College of Rheumatology classification criteria. For all patients included in this study, we collected the following information: age, gender, duration of disease, exposure to sun, treatment, origin of patients according to geographic location, history of lupus nephritis, vitamin D and calcium supplementation, doses and duration of supplements and 25-hydroxyvitamin D [25(OH)D] levels. This study was approved by scientific committee of Faculty of Medical Laboratory Sciences, University of Khartoum. Informed consent was obtained from all individual participants included in the study.

The normal range of vitamin D is measured as nanograms per milliliter (SI unit = nmol/L). Many experts recommend a level between 20 and 40 ng/ml (50-100 nmol/L). Others recommend a level between 30 – 50 ng/ml (75-125 nmol/L)[20]. Target blood levels of vitamin D are currently thought to be 30—40 ng/ml (75-100 nmol/L). Daily doses of 1000-2000 IU achieve this level in most patients, and 2000 IU/day is considered safe. A day in the sun produces about 10,000 IU, but after about 20,000 IU the sun destroys vitamin D in the skin, a self-regulating mechanism that prevents toxicity [13].

Group I (patients):

This group was further subdivided according to the presence of nephritis into two subgroups:

A: 35 patients without lupus nephritis (LN).

B: 25 patients with LN.

Group II (control):

A total of 60 normal healthy subjects were included as a control group; they were matched for age and sex with SLE patients.

Blood sample were collected in plain containers, and then subjected to centrifugation to obtain serum. The serum obtained was stored at -20°C until analysis.

Measurement of Vitamin D:

Serum 25(OH) D was measured using ELISA technique (KAP 1971: 96 test, immunoenzymatic assay [DIAsource company], Belgium), which evaluates the total vitamin D levels, both the D2 and D3 forms. It measures both the D2 and D3 derivatives of 25(OH)D in serum. Vitamin D deficiency was defined as serum 25(OH)D level <25 nmol/L; insufficient if 25(OH)D 25-74 nmol/L; sufficient if 25(OH)D 75-250 nmol/L; and potential toxicity if 25(OH)D >250 nmol/L.

Statistical analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS) Version 20. The results expressed as Mean \pm SD, frequency, and percentage. Independent T-test was using to compare vitamin D level between case and control groups. One- Way ANOVA was obtained to compare vitamin D level among different type of vitamin D and calcium supplementation and different doses of supplementation. Chi-square test was performed to compare frequency and percentage of vitamin D status among case and control group. Pearson correlation was obtained to study the relation between vitamin D level and study variables (age, duration of disease, and duration of vitamin D and calcium supplement).

3. Results

A total of 120 participants, 60 patients were satisfied the criteria for inclusion and 60 healthy subjects as control, the age and gender were matched in both groups (case and control). The mean age in case group was 31 \pm 9 and in control group was 29 \pm 8. Among case group 88% were females and 12% were males, while 90% of control group were females and 10% were males (Figure 1).

According to obtained demographics data we found that the percentage of the origin of the sixty SLE patients were: 55% from north, 18% from west, 16.7% from middle, 6.7% from the south, and 3.3%, from east of Sudan. The results show that vitamin D levels were significantly increased in case group compared to control group (p-value 0.002) (Table 1).

On assessing vitamin D status for case group who took different doses of vitamin D and calcium supplement the results were as follows: 30 patients (50%) had insufficient levels, 19 patients (31.7%) had sufficient level, 7 patients (11.7%) were in toxic level, and 4 patients (6.7%) were in deficient level; control group show these results 33 subjects (55%) insufficient level, 19 subjects (31.7%) have sufficient level, 8 subjects (13.3%) were in deficient level, and no one were in toxic level (P-value: 0.037) (Table 2).

Vitamin D levels were significantly higher in patients who took supplement; depending on supplement type; compared to those who did not take any type of supplement (41.62±27.29 nmol/L). Patients who took vitamin D and calcium (233.85±128.44 nmol/L; P-value: 0.000), followed by patients who use vitamin D supplement only (176.15±139.70 nmol/L; P-value: 0.006). In addition, patients who took calcium supplement only (80.89±34.11 nmol/L; P-value: 0.022), (Table 3).

Vitamin D levels were positively correlated to the dose of vitamin D supplement taken, when compare to patients who not took any dose (55.36±35.00 nmol/L), patients who took high dose (50000-60000 IU) had mean concentration of 338.±114.86 nmol/L (P-value: 0.000), followed by patients took medium dose level (400-1000 IU) with mean of 196.00±102.05 nmol/L (P-value: 0.000), then patients who used low dose (100 IU) had level of 83.50±33.87 nmol/L; (P-value: 0.002) (Table 4).

The difference in Vitamin D levels among two subgroup SLE and LN patients was insignificant; P-value: 0.345 (Table 5).

There was no association between vitamin D level and duration of disease [(R: 0.010, P-value: 0.939). However, very low levels as 20 & 4 nmol/L were found in two subjects who were newly diagnosed with SLE (one was diagnosed three months ago and the other was diagnosed only 2 weeks) Vitamin D level was positively correlated with duration of supplementation in months [(R: 0.374, P-value: 0.003) (Figure 3).

Table 1: Shows the mean concentration comparison of Vitamin D level among case and control group

Variable	Group	Mean ±SD	P-value
VitaminD level (nmol/L)	Case	105.24±13.93	0.002
	Control	59.12±4.06	

Table 2: Shows the Vitamin D status among case and control group

Vitamin D status		Group		Total
		Case	Control	
Deficient	Count	4	8	12
	%	6.70%	13.30%	10.00%
Insufficient	Count	30	33	63
	%	50.00%	55.00%	52.50%
Sufficient	Count	19	19	38
	%	31.70%	31.70%	31.70%
Potential Toxicity	Count	7	0	7
	%	11.70%	0.00%	5.80%
Total	Count	60	60	120
P-value		0.037		

Table 3: Shows the mean comparison of Vitamin D levels according to type of supplement

Vitamin D and calcium supplement	Mean ±SD (nmol/L)	P-value
Without supplement	41.62±27.29	
vitamin D only	176.15±139.70	0.006
Vitamin D and calcium	233.85±128.44	0.000
calcium only	80.89±34.11	0.022

Table 4: Shows the mean comparison of Vitamin D levels according to the dose of supplement

Dose group	Mean ±SD (nmol/L)	P-value
No supplement	55.36±35.00	-
high(50000-60000 IU)	338.50±114.86	0.000
medium(400-1000 IU)	196.00±102.05	0.000
low (100IU)	83.50±33.87	0.002

Table 5: Shows the mean comparison of Vitamin D level among subgroup patients

Variable	Disease	Mean ±SD (nmol/L)	P-value
Vitamin D level (nmol/L)	SLE	100.88±17.10	0.345
	LN	111.34±23.71	

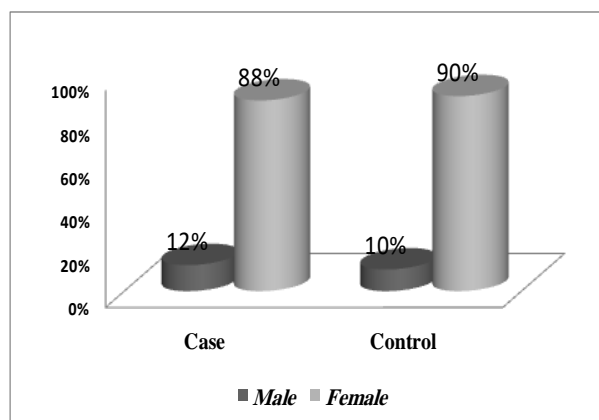


Figure 1: shows gender distribution among the study group

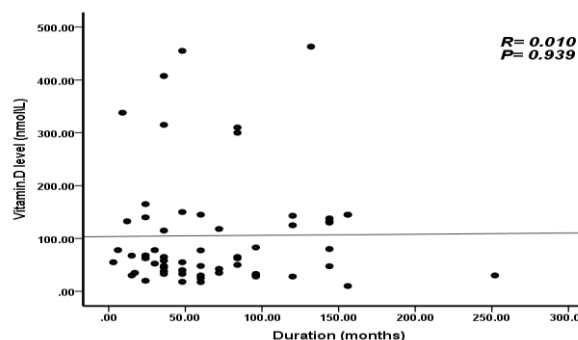


Figure 2: shows the correlations between duration of disease and vitamin D levels

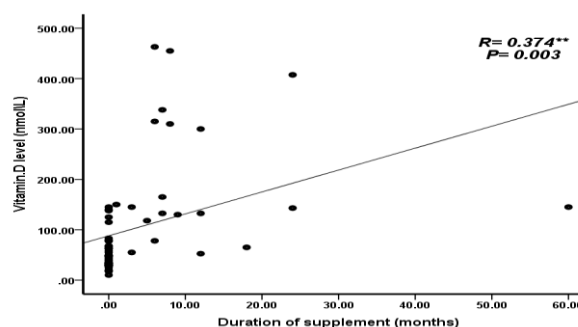


Figure 3: shows the correlations between duration of supplements and vitamin D levels

4. Discussion

1, 25-Dihydroxyvitamin D (calciferol) is the most active form of vitamin D and considered as an essential steroid hormone. It has a role in bone homeostasis via calcium and phosphorus metabolism [1-4]. Systemic lupus

erythromatosis (SLE) is a chronic autoimmune disease that attacks healthy tissue of almost any organ system [9, 10].

Many studies have shown that there is an association between vitamin D and SLE. SLE patients are more likely to have lower levels of vitamin D. In addition, the disease severity has been linked to low vitamin D levels. This may be due to SLE patients' sensitivity to the sun and are advised to avoid being outdoors [2, 13, 15].

Therefore, this study aims to evaluate vitamin D levels among SLE patients and correlate these levels to duration of disease, vitamin D/calcium supplement usage, doses and duration of supplementation.

The present study revealed that SLE and LN patients had higher level of vitamin D level as compared to control group, this result disagree with Suzan M. Attar *et al* [14] and the previous studies conducted on SLE patients who were newly diagnosed and did not receive vitamin D and/or calcium supplement, all these studies reported that vitamin D level was lower in SLE and LN compared to control.

Our study shows agreement with study conducted by Charlene Laino *et al* [19], which reported that using high dose of vitamin D supplement (100,000 IU) led to increased blood vitamin D level.

Although in our case vitamin D level was increased; this may be due to using vitamin D and calcium supplement in different doses and duration. The decreased levels in control group was because most people who appear as healthy do get exposed to the sun for proper period of time or in preferred time. Moreover, low control levels could be due to: Sudanese cultural dresses (covering all of their body except hands and faces), hidden diseases that related to absorption and metabolism problem and Sudanese dark skin, which could decrease effect of sun in vitamin D synthesis. However, this agree with study performed by Osama Eltayeb *et al* [21], which measured vitamin D levels among healthy Sudanese volunteers in Khartoum revealing that low vitamin D level was common Sudanese individuals who appear to be healthy.

No evidence from this study showed that decrease in disease activity was related to vitamin D supplementation; and this agree with a systematic review done by Martin A. Krieger *et al* [22], who mentioned that prospective interventional and evidence data in human was still lacking.

A large proportion (n=53, 58.3%) of patients were receiving vitamin D and calcium supplementation. Although our study was not initially designed to determine the indication for prescription of vitamin D supplementation, it was likely that physician were influenced by the high prevalence of corticosteroid and anti-malarial use, and associated osteoporosis risk in SLE patients.

As all patients were on HCQ and corticosteroids therapy, so their relationship to serum vitamin D could not be determined.

5. Acknowledgment

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6. Conclusion

25 (OH) D deficiencies are common in patients with SLE who are not supplemented with vitamin D /calcium. Although no significance relationship existed between vitamin D level and subgroup of patients (SLE and LN), it was observed that vitamin D level is lower in SLE patients than LN patients and this indicate that vitamin D level not associated to disease activity.

Measurement of vitamin D level is necessary in patients who took high dose of vitamin D supplement to avoid the danger of reaching the toxic level.

For more convenient results such study should be conducted on newly diagnosed SEL subject who did not receive any vitamin D supplement yet and the disease activity should be measured concurrently to assess the correlation.

Assessment of vitamin D levels should be done for newly diagnosed SLE patients and monitoring until achieving sufficient levels then followed by regular recommended daily dose.

The difficult access to Sudanese SLE patients was one of the limitations of this study, since the disease is still considered to be unknown among Sudanese. Therefore, spreading awareness about SLE may assist researches to find access to more data on this subject.

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