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ORIGINAL ARTICLE

Calcium and vitamin D supplement intake may increase arterial stiffness in systemic lupus erythematosus patients

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Abstract

Objectives Low serum levels of 25-hydroxyvitamin D (25(OH)D) have been associated with a higher frequency of risk factors and cardiovascular disease. The aim of this study is to evaluate the association of 25(OH)D, cardiovascular risk factors, and subclinical atherosclerosis in systemic lupus erythematosus (SLE) patients.

Method Forty-seven female SLE patients were studied. Data collected included demographics, SLE activity, disease damage, cardiovascular risk factors, and markers of subclinical atherosclerosis. Patient treatments and vitamin D and calcium supplementation (VitD-Ca) were recorded. Vitamin D deficiency was defined as serum 25(OH)D < 50 nmol/l measured by ultra-high-performance liquid chromatography. Atherosclerosis was assessed by measuring the carotid-femoral pulse wave velocity (PWV) by Doppler velocimetry and intima-media thickness (IMT) by B-mode ultrasound scanning.

Results 61.7% of patients were vitamin D deficient with a mean level of 31.91 ± 10.21 nmol/l. Serum vitamin D concentration was significantly higher in the 23 patients taking VitD-Ca supplements than that in patients not supplemented ($p = 0.004$). No significant association was found between 25(OH)D serum levels and cardiovascular risk factors, disease activity, or different treatments for SLE. A significant positive correlation was found between 25(OH)D levels, PWV ($p = 0.02$), and IMT ($p = 0.01$); moreover, patients taking VitD-Ca supplements presented an increased arterial stiffness.

Conclusion Patients with arterial stiffness showed higher levels of serum vitamin D and most of them were on VitD-Ca supplements. Although prospective studies with a larger number of patients and follow-up are needed, our findings suggest that VitD-Ca supplementation may have effects on SLE patients' arterial stiffness.

Keywords 25-Hydroxyvitamin D · Subclinical atherosclerosis · Systemic lupus erythematosus · Vit D-calcium supplementation

Introduction

Systemic lupus erythematosus (SLE) is a systemic inflammatory disease with a bimodal mortality pattern, the second peak being due to cardiovascular disease [1]. SLE patients have a four to 10-fold higher risk of cardiovascular diseases than the general population which is due to accelerated atherosclerosis [2]. While preliminary studies suggest that traditional cardiovascular risk factors may play a role, they do not seem to explain fully the increased prevalence of atherosclerosis [3, 4]. Therefore, non-traditional cardiovascular risk factors and other factors related to SLE disease such as systemic chronic inflammation, immunological disturbances, clinical manifestations, and side effects of therapies for SLE may be additional factors involved in the premature development of atherosclerosis. Indeed, it has been shown that, as for diabetes mellitus, SLE itself is an independent risk factor for the development of atherosclerosis [5].

Vitamin D has been known for some time to be a hormone that regulates bone homeostasis via calcium and phosphorus metabolism. Recently, other new functions have been

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discovered including protective actions against autoimmune diseases, cancer, infections, and cardiovascular diseases [6]. The vitamin D receptor has been identified in cells of both the immune system (macrophages, dendritic cells, antigen-presenting cells, T and B cells) and the cardiovascular system (cardiac myocytes, endothelial cells, and vascular smooth muscle cells) [6, 7]. Epidemiological studies have demonstrated that vitamin D deficiency is associated with cardiovascular risk factors and atherosclerosis in the general population. The National Health and Nutrition Examination Surveys and other cross-sectional cohort studies have found a relationship between vitamin D deficiency and cardiovascular risk factors such as hypertension, diabetes mellitus, obesity, hypertriglyceridemia, and microalbuminuria [8–11]. Furthermore, lower serum 25-hydroxyvitamin D (25(OH)D) levels are associated with the presence of subclinical cardiovascular disease, including endothelial dysfunction, carotid intima-media thickness, and coronary artery calcification [12, 13], as well as, with an increased prevalence of cardiovascular events including myocardial infarction, congestive heart failure, angina, stroke, and peripheral arterial disease [14–17].

Vitamin D deficiency is more common in patients with SLE than in age-and gender- matched controls [18, 19]. In the Carolina Lupus Inception Cohort Study, overall, 67% of the subjects were vitamin D deficient [18]. Lower levels of vitamin D have been associated with SLE disease activity, high body mass index, serum creatinine, nephritis, daily sunscreen use, lack of sun exposure, hydroxychloroquine intake, cumulative steroids dose, and low bone mineral density [18, 20–22]. The presence of low levels of vitamin D, as noted earlier, has been associated with cardiovascular risk factors and subclinical and cardiovascular disease. The aim of this study is to evaluate in SLE patients whether the levels of vitamin D are associated with cardiovascular risk factors, markers of subclinical atherosclerosis, and SLE-related factors.

Materials and methods

Study population

This cross-sectional study included 47 consecutive SLE patients attending scheduled appointments in our outpatients' Autoimmune Diseases Unit at our hospital. Inclusion criteria were non-pregnant women over 18 years of age who fulfilled at least four ACR revised 1997 classification criteria for SLE. The study was approved by the Research Ethics Committee of the Hospital Universitario Puerta de Hierro and written informed consent was obtained from all participants.

Patients were assessed for comorbidity, traditional and non-traditional cardiovascular risk factors, SLE-related factors, and subclinical atherosclerosis status. A full clinical history and physical examination including weight, height, body mass index, waist circumference, and blood pressure was undertaken. The mean disease duration, demographics, and clinical data were obtained from the medical records.

None of the patients had clinical coronary heart disease or peripheral arterial disease. Four patients presented symptomatic cerebral vascular disease due to antiphospholipid syndrome with no arterial plaque detection.

Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or the patient being on antihypertensive treatment. Diabetes mellitus was considered when the patient was treated with oral agents or insulin or if fasting glucose was ≥ 126 mg/dl, while impaired fasting glucose was defined when values were between 100 and 125 mg/dl. Hyperlipidemia was defined as total cholesterol ≥ 190 mg/dl or LDL cholesterol ≥ 115 mg/dl or triglycerides ≥ 150 mg/dl or lipid-lowering therapy. Family history of cardiovascular disease was considered if the patient had a first-degree relative who had suffered a heart attack or stroke before age 55 (men) or 65 (women). The presence of metabolic syndrome was calculated according to the definitions used by the Adult Treatment Panel III (ATP III).

The activity of SLE was assessed by the SLE Disease Activity Index (SLEDAI), with inactive disease defined as SLEDAI ≤ 4 and organ damage by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index, defining no organ damage when SLICC/ACR = 0. The clinical manifestation of SLE (cutaneous, visceral, or mixed) was considered.

Fasting blood samples were taken for measurement of lipid profiles, glucose, hemoglobin A1c, uric acid, creatinine, urea, and inflammatory markers such as ultra-sensitive C-reactive protein (hsCRP), homocysteine, D-dimer, and fibrinogen.

The following SLE-related factors were considered positive: ANA (immunofluorescence) > 1:40, anti-dsDNA antibodies (ELISA) > 15 U/ml, anti-ENA antibodies (ELISA) > 10 U/ml, lupus anticoagulant (Russell's viper venom; confirmatory ratio > 1.12), anti-cardiolipin antibodies (ELISA) > 18 U/ml, and anti-β2 glycoprotein 1 antibodies (ELISA) > 8 U/ml. Other lupus-related parameters, such as full blood counts, erythrocyte sedimentation rate, and complement levels (C3 and C4), were determined. Information concerning the treatment patients was receiving (steroids and dose, hydroxychloroquine, immunosuppressive, vitamin D plus calcium supplements, statins, antihypertensive, and antiplatelet drugs) in 3 months before their recruitment was recorded.

The 25(OH)D levels in the serum were determined by ultra-high-performance liquid chromatography (UPHLC) for all patients. The validity of the methodology was tested using external samples from an international quality control program (Vitamin D External Quality Assessment Schema-DEQAS, Charing Cross Hospital, London, UK). According to current recommendations,

serum 25(OH)D levels < 50 nmol/l (20 ng/ml) were defined as vitamin D deficiency[6].

Subclinical atherosclerosis assessment

Pulse wave velocity measurement

Arterial stiffness was assessed by measuring the carotid-femoral pulse wave velocity (PWV) by Doppler velocimetry and simultaneous electrocardiogram. Simultaneous recordings of the arterial flow waves from the right common carotid artery and the right femoral artery were made by a bidirectional transcutaneous Doppler velocimeter device using an 8-MHz probe. After waveform collection, the distance between the carotid and femoral sampling sites was measured with a standard tape measure. PWV was calculated in meters per second by dividing the distance by the time component.

Carotid atherosclerosis

Carotid atherosclerosis was determined by assessing the intima-media thickness (IMT) and carotid plaque using a B-mode ultrasound scanner (Phillips IU22) equipped with eco-Doppler and IMT automatic quantification. Sonographers scanned the right and left common carotid arteries, the carotid bulb, and the first 15 mm of the internal and external carotid arteries.

All measurements of IMT were made in the longitudinal plane at the point of the maximum thickness on the far wall of the common carotid artery along a 1-cm section of the artery proximal to the carotid bulb. Values from each location were then averaged to produce an overall measure of IMT. The presence of carotid plaque was defined as a distinct area protruding into the vessel lumen.

Acquisition and analysis of both PWV and IMT data of all patients were performed by the same experienced operator who was blinded to the subject clinical features.

Statistical analysis

Data were presented as the mean ± standard deviation (SD) or as percentages for qualitative variables. The Kolmogorov test was used to evaluate the distribution. For quantitative data with a non-Gaussian distribution, statistical analysis was performed with the nonparametric Mann-Whitney U test and when a normal distribution was followed, Student's t test was carried out. The χ^2 test (with two-sided Fisher's exact test) was used to compare categorical variables and Yates's correction applied when the frequencies found were less than 5. Linear and logistic regression analyses were used to investigate associations between 25(OH)D and subclinical atherosclerosis (PWV, IMT).

For all analyses, significance was defined as a P value of less than 0.05. Statistical analysis was performed using SPSS software.

Results

Characteristics of patients

Forty-seven women patients with SLE were recruited. The clinical characteristics and cardiovascular risk factors of the patients included in the study are shown in Table 1. The median age was 48.8 years (21–65) with a mean disease duration of 10.85 ± 7.9 years with 22 patients (40.8%) having had the disease for more than 10 years. All patients were Caucasian.

Thirty-six patients had an inactive disease defined as SLEDAI ≤ 4, nine patients had a SLEDAI of between 5 and 26, and the disease activity was unknown in two patients. Cutaneous manifestations were present in 41 patients (87.2%) and 14 patients (29.8%) had lupus nephritis. At the time of blood extraction for this study, 19 patients had serological activity based on the presence of positive antiDNA antibodies and/or low complement levels. Fourteen patients (29.8%) fulfilled the laboratory criteria for antiphospholipid syndrome but only six met the clinical criteria.

The organ damage, assessed by the SLICC/ACR Damage Index, was between 1 and 6 for 37 patients while the other 10 patients had no organ damage (SLICC/ACR = 0).

Four patients (8.5%) were on no medication and 41 patients (87.2%) were taking antimalarials regularly (predominantly hydroxychloroquine), either as the only treatment (16 patients) or in combination with some immunosuppressive drug. Twenty-six patients (55.3%) were on corticosteroid therapy with the mean cumulative dose of steroids being 26.61 ± 20.75 g. Seventeen patients (36.2%) were also taking immunosuppressive drugs (mycophenolate mofetil $n = 7$, azathioprine $n = 5$, methotrexate $n = 5$).

Prevalence of vitamin D deficiency

The mean value of 25(OH)D was 45.95 ± 20.79 nmol/l. Levels of 25(OH)D ≥ 50 nmol/l were detected in 18 patients (38.3%) with a mean of 68.57 ± 10.99 nmol/l, and 29 patients (61.7%) were vitamin D deficient with a mean level of 31.91 ± 10.21 nmol/l.

Vitamin D and cardiovascular risk factors

Higher levels of 25(OH)D were seen in hypertensive patients compared with non-hypertensive patients (57.39 ± 23 nmol/l vs 41.10 ± 18.04, $p = 0.012$), and only 28.57% of the hypertensive patients were vitamin D deficient compared with 75.75% of non-hypertensive patients ($p = 0.007$). There was

Table 1 Clinical characteristics and disease-specific features of SLE patients

	Patients ($n = 47$)
Median age (years)	48.8 (21–65)
Mean disease duration (years)	10.85 ± 7.9
Mixed (visceral & cutaneous) disease manifestations (%)	78.72 ($n = 37$)
Positive ANA antibodies (%)	95.74 ($n = 45$)
Positive anti-dsDNA antibodies (%)	31.91 ($n = 15$)
Hypocomplementemia (%)	10.64 ($n = 5$)
Antiphospholipid syndrome (%)	12.76 ($n = 6$)
SLEDAI > 4 (%)	19.15 ($n = 9$)
SLICC/ACR ≥ 1(%)	78.72 ($n = 37$)
Current treatment (%)	
Non-treatment	8.51 ($n = 4$)
Antimalarials only	34.04 ($n = 16$)
Antimalarials + steroids + immunosuppressive drugs	29.79 ($n = 14$)
Antimalarials + steroids	21.28 ($n = 10$)
Antimalarials + immunosuppressive drugs	2.13 ($n = 1$)
Steroids + immunosuppressive drugs	4.26 ($n = 2$)
Classic cardiovascular risk factor (%)	
Arterial hypertension	29.79 ($n = 14$)
Diabetes mellitus	4.26 ($n = 2$)
Hyperlipidemia	40.42 ($n = 19$)
Tobacco habit	21.28 ($n = 10$)
Metabolic syndrome	25.53 ($n = 12$)
Family history of CVD	27.66 ($n = 13$)

Data were presented as percentages for categorical data, as the mean ± standard deviation or as the median (range). *SLEDAI* SLE Disease Activity Index, *SLICC/ACR* Systemic Lupus International Collaborating Clinics Organ Damage Index, *ANA* antinuclear antibodies, *CVD* cardiovascular disease

no significant association between serum level 25(OH)D and other cardiovascular risk factors (Table 2). The 15 patients included in the study taking statins as a treatment for their cardiovascular risk factors exhibited a trend towards higher levels of 25(OH)D (51.93 ± 22.9 nmol/l vs 43.15 ± 19.44 nmol/l).

In relation to the non-traditional CV risk factors, an inverse correlation between 25(OH)D and hsCRP ($r = -0.29$; $p = 0.05$) as well as D-dimer ($r = -0.34$; $p = 0.02$) was observed while patients did not show significant differences in homocysteine or fibrinogen levels.

Vitamin D and SLE-related factors

No correlations were found between 25(OH)D levels and neither the presence of antiDNA antibodies, C3 and C4 levels, and antiphospholipid antibodies nor with the disease activity (Table 3). However, a trend towards higher levels of 25(OH)D was found in patients with organ damage assessed by SLICC/ACR (48.75 ± 21.45 vs 35.59 ± 14.78, $p = 0.08$). No significant association was found between vitamin D levels and different treatments the patients were receiving for SLE.

Vitamin D and subclinical atherosclerosis

Aortic stiffness was measured by PWV and the mean of PWV in our SLE patients was 7.22 ± 2.30 m/s. The results were adjusted for age and blood pressure, based on normal standards published by the European Society of Cardiology [23]. SLE patients were divided into two groups, one with PWV in the normal range and the other with PWV in the pathological range. In our study, an intima-media thickness (IMT) cut-off was established defined by the median IMT obtained from our patient group. This allowed our SLE patients to be divided into two groups, one with normal, i.e., IMT < 0.53 mm (22 patients) and the other with IMT ≥ 0.53 mm (23 patients). The PWV and the IMT were not determined for two patients.

Pathological PWV was found in 17 patients (37.8%) and 28 patients (62.2%) had a normal PWV. No significant association was found between 25(OH)D deficiency and pathological PWV nor between 25(OH)D levels and pathological IMT (Table 4). Nevertheless, the patients with normal PWV or normal IMT most often presented vitamin D deficiency (20 out of 28 patients with normal PWV and 16 out of 22 patients with normal IMT). Furthermore, a significant positive correlation was found between 25(OH)D levels and PWV ($r = 0.35$, $p =$

Table 2 Association between 25(OH)D concentration and the presence of cardiovascular risk factors

Cardiovascular risk factors	Presence	Absence	*p* value
Arterial hypertension	57.39 ± 23.00 (*n* = 14)	41.10 ± 18.04 (*n* = 33)	*0.01*
Tobacco	41.29 ± 20.45 (*n* = 10)	47.21 ± 20.98 (*n* = 37)	0.43
Diabetes mellitus	30.40 ± 19.09 (*n* = 2)	46.64 ± 20.79 (*n* = 45)	0.28
Impaired fasting blood glucose	38.35 ± 22.12 (*n* = 10)	48.64 ± 20.24 (*n* = 37)	0.19
Hyperlipidemia	49.41 ± 21.25 (*n* = 19)	43.61 ± 20.53 (*n* = 28)	0.35
Body mass index ≥ 30 kg/m^2	48.48 ± 22.25 (*n* = 27)	42.54 ± 18.67 (*n* = 20)	0.34
Waist circumference ≥ 88 cm	43.03 ± 20.68 (*n* = 7)	46.47 ± 21.04 (*n* = 40)	0.69
Metabolic syndrome	46.86 ± 24.99 (*n* = 12)	45.64 ± 19.56 (*n* = 35)	0.86
Uric acid > 6 mg/dl	53.91 ± 22.68 (*n* = 10)	43.80 ± 20.05 (*n* = 37)	0.18
Creatinine clearance ≥ 60 ml/min	50.03 ± 18.47 (*n* = 10)	44.85 ± 21.48 (*n* = 37)	0.49
Microalbumin/creatinine > 299 mg/g	38.50 ± 17.58 (*n* = 5)	45.94 ± 22.22 (*n* = 28)	0.49
hsCRP > 1 mg/l	44.23 ± 22.42 (*n* = 20)	47.25 ± 19.05 (*n* = 27)	0.63
Homocysteine > 12.5 μmol/l	44.29 ± 19.64 (*n* = 14)	44.84 ± 20.12 (*n* = 33)	0.93
D-Dimer > 0.5 μg/l	41.82 ± 18.43 (*n* = 17)	48.29 ± 21.98 (*n* = 30)	0.31
Fibrinogen > 450 mg/dl	36.95 ± 19.52 (*n* = 9)	48.09 ± 20.76 (*n* = 38)	0.15
Family history of CVD	49.18 ± 24.63 (*n* = 13)	44.71 ± 19.41 (*n* = 34)	0.52
Cardiovascular therapy			
Antihypertensive	51.60 ± 21.39 (*n* = 11)	44.23 ± 20.61 (*n* = 36)	0.31
Statins	51.93 ± 22.99 (*n* = 15)	43.15 ± 19.44 (*n* = 32)	0.18
Acetylsalicylic acid	45.99 ± 22.40 (*n* = 19)	45.93 ± 20.06 (*n* = 28)	0.99

Data were presented as the mean ± standard deviation (SD). Statistical significant differences in the serum 25(OH)D concentration between patients with or without the presence of cardiovascular risk factors were highlighted in italics. *CVD* cardiovascular disease

0.02) and IMT ($r = 0.36$, $p = 0.01$), which indicates that patients with higher levels of 25(OH)D had higher PWV and IMT.

Effects of vitamin D and calcium supplementation

Twenty-three patients (48.9%) received vitamin D and calcium (VitD-Ca) supplements, a cholecalciferol dose of 400 to 800 IU per day combined with at least 500 to 1000 mg of calcium. The 25(OH)D levels in these patients were significantly higher than those in patients not supplemented (54.54 ± 21.8 nmol/l vs 37.72 ± 16.31 nmol/l, $p = 0.004$). Only nine of the 23 patients taking VitD-Ca supplements were vitamin D deficient, in contrast to the 20 vitamin D deficient patients in the 24 not taking VitD-Ca supplements (Fig. 1).

Table 3 The relationship between serum 25(OH)D concentration and SLE-related factors

SLE-related factors	Presence	Absence	*p* value
Positive ANA	45.58 ± 21.15 (*n* = 45)	54.8 ± 8.70 (*n* = 2)	0.53
Positive anti-dsDNA antibodies	46.21 ± 22.67 (*n* = 15)	45.83 ± 20.24 (*n* = 32)	0.96
Positive anti-ENA antibodies	43.40 ± 21.18 (*n* = 21)	46.41 ± 20.04 (*n* = 26)	0.63
Hypocomplementemia	36.02 ± 17.94 (*n* = 11)	48.89 ± 21.18 (*n* = 35)	0.08
SLEDAI > 4	48.71 ± 18.96 (*n* = 9)	44.71 ± 21.52 (*n* = 36)	0.61
SLICC/ACR ≥ 1	48.75 ± 21.45 (*n* = 37)	35.59 ± 14.78 (*n* = 10)	0.08
Antiphospholipid antibodies	48.35 ± 21.20 (*n* = 10)	45.86 ± 20.96 (*n* = 37)	0.74
Antiphospholipid syndrome	53.68 ± 22.83 (*n* = 6)	44.82 ± 20.54 (*n* = 41)	0.33
Current treatment			
Non-treatment	40.40 ± 20.53 (*n* = 4)		
HCQ only	49.13 ± 21.81 (*n* = 16)		
Immunosuppressive therapy ± HCQ	44.90 ± 20.74 (*n* = 27)		

Data were presented as mean ± standard deviation (SD). *ANA* antinuclear antibodies, *SLEDAI > 4* SLE Disease Activity Index > 4 (active disease), *SLICC/ACR ≥ 1* Systemic Lupus International Collaborating Clinics Organ Damage Index ≥ 1 (organ damage presence) and *HCQ* hydroxychloroquine

Table 4 The relationship between serum 25(OH)D concentration and subclinical atherosclerosis

	Serum 25(OH)D (nmol/l)	*p* value
Normal PWV ($n = 28$)	44.94 ± 20.17	0.50
Pathological PWV ($n = 17$)	49.25 ± 20.87	
IMT < 0.53 mm ($n = 22$)	43.29 ± 20.72	0.22
IMT ≥ 0.53 mm ($n = 23$)	50.87 ± 19.87	

Serum 25(OH)D concentrations were presented as the mean ± standard deviation (SD). SLE patients were classified according to the results of PWV adjusted for age and blood pressure and categorized IMT measurements. *PWV* pulse wave velocity, *IMT* intima-media thickness

No association was observed between VitD-Ca supplement intake and cardiovascular risk factors or SLE-related factors. However, a near significant statistical correlation was found between arterial stiffness and 25(OH)D levels in VitD-Ca-supplemented patients ($r = 0.36$, $p = 0.09$) and 11 out of 17 patients (64.7%) with pathological PWV were taking VitD-Ca supplements.

As results on 25(OH)D and subclinical atherosclerosis obtained were apparently paradoxical and contradicted some previous studies, the relationship between higher arterial stiffness and the VitD-Ca supplementation becomes an interesting point to check. As the patients were not only taking vitamin D supplements but also calcium supplements, it was hypothesized that the greater arterial stiffness may be due to the levels of serum calcium in the patients.

Analyzing the serum calcium levels in relation to arterial stiffness and IMT, a significant positive correlation between IMT and serum calcium concentration was found ($r = 0.36$, $p = 0.01$), as well as a tendency for greater arterial stiffness with increased serum calcium levels ($r = 0.26$, $p = 0.08$). At the same time, it was observed that patients with pathological PWV exhibited a near statistically significant trend of a greater concentration of serum calcium than patients with normal PWV (9.53 ± 0.43 vs 9.23 ± 0.52, $p = 0.057$) (Table 5). Although, higher levels of serum calcium were not present in patients taking VitD-Ca supplements (9.35 ± 0.50 vs 9.3 ± 0.52 $p = 0.73$), supplemented patients with pathological IMT had a statistically significant greater serum calcium level (9.55 ± 0.39 vs 9.13 ± 0.53, $p = 0.041$) and a trend towards a higher arterial stiffness with higher serum calcium levels (9.55 ± 0.4 vs 9.16 ± 0.53, $p = 0.057$).

Discussion

Our results show a low serum 25(OH)D concentration, with about 62% of patients with a level lower than 50 nmol/l, despite the fact that our population resides in a Southern European country with a high number of sunny days. This is in agreement with previous studies carried out in Spain, describing that 75–85.5% of patients had levels lower than 30 ng/ml and 15–38.2% lower than 10 ng/ml [24, 25]. These results were also similar to those seen in SLE patients from South Carolina [18], Manchester [26] and Large International Inception Cohort [27] with 52–72% vitamin D deficient patients. Most, published data agree that there is a high prevalence of vitamin D deficiency in SLE patients [28]. In our study, approximately 50% of patients were taking VitD-Ca supplements, following the recommendations of clinical guidelines for the prevention and treatment of osteoporosis, and the 25(OH)D levels in these patients were significantly higher than those in not supplemented patients. Our findings are consistent with other studies that have shown that vitamin D supplements increase serum vitamin D levels [24, 29].

Regarding the association of vitamin D and cardiovascular risk factors, our results showed that hypertensive patients had significantly higher levels of 25(OH)D compared with non-hypertensive patients. We have not found any significant association between serum 25(OH)D level and other cardiovascular risk factors. However, data from a large international inception cohort have shown that those in the lower 25(OH)D quartiles were more likely to have hypertension and hyperlipidemia compared with those in the highest quartile, even after adjusting for age, season, sex, race, country/

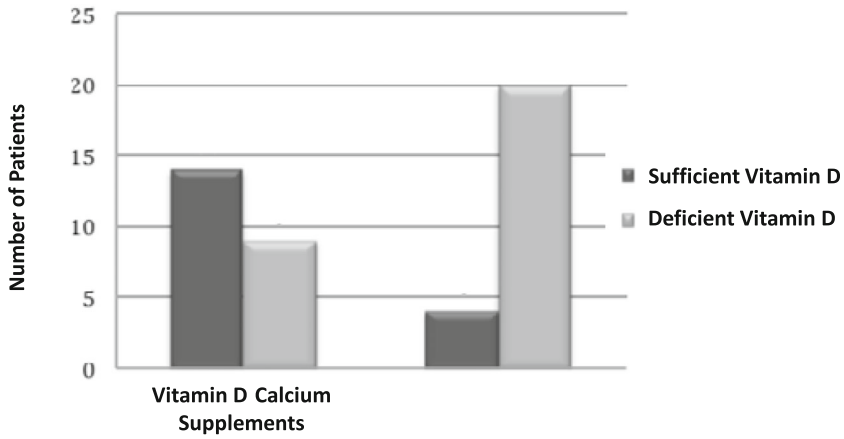

Fig. 1 Vitamin D deficiency. Gray bars represent SLE patients with deficient serum 25(OH)D concentration (< 50 nmol/l). Patients have been grouped by the vitamin D and calcium supplement intake

Table 5 Association of serum 25(OH)D and calcium concentrations with subclinical atherosclerosis according to patients VitD-Ca supplementation

	Serum 25(OH)D concentration (nmol/l)		
Vit D-Ca supplements	Normal PWV ($n = 28$)	Pathological PWV ($n = 17$)	
No supplements	37.25 ± 12.05 ($n = 16$)	39.58 ± 20.90 ($n = 6$)	0.75
With supplements	54.56 ± 24.39 ($n = 12$)	54.53 ± 19.79 ($n = 11$)	0.99
	IMT < 0.53 mm ($n = 22$)	IMT ≥ 0.53 mm ($n = 23$)	
No supplements	38.57 ± 14.84 ($n = 11$)	40.32 ± 17.61 ($n = 11$)	0.80
With supplements	48.01 ± 25.14 ($n = 11$)	60.53 ± 17.16 ($n = 12$)	0.17
	Serum calcium concentration (mg/dl)		
Vit D-Ca supplements	Normal PWV ($n = 28$)	Pathological PWV ($n = 17$)	*p* value
No supplements	9.26 ± 0.53 ($n = 16$)	9.48 ± 0.53 ($n = 6$)	0.39
With supplements	9.16 ± 0.53 ($n = 12$)	9.55 ± 0.40 ($n = 11$)	0.057
	IMT < 0.53 mm ($n = 22$)	IMT ≥ 0.53 mm ($n = 23$)	
No supplements	9.26 ± 0.51 ($n = 11$)	9.35 ± 0.55 ($n = 11$)	0.67
With supplements	9.13 ± 0.53 ($n = 11$)	9.55 ± 0.39 ($n = 12$)	*0.041*

Serum 25(OH)D and calcium concentrations were presented as the mean ± standard deviation (SD) considering the patients intake of VitD-Ca supplements. SLE patients were classified according to results of PWV adjusted for age and blood pressure and categorized IMT measurements. Statistical significant differences in calcium level between patients were highlighted in italics. *PWV* pulse wave velocity, *IMT* intima-media thickness

location, and body mass index [27]. Recently, vitamin D deficiency has been associated with non-dipper hypertension in women with SLE [30]. Our results could be explained by a higher number of hypertensive patients taking VitD-Ca supplements.

There are several studies that describe an inverse relationship between 25(OH)D levels and disease activity [21, 26, 27]. However, other studies including ours find no correlation between 25(OH)D levels and disease activity even though it must be noted that in our study based on a relatively small number of patients, only nine patients had a SLEDAI ≥ 5 [24, 25, 29, 31].

Vitamin D deficiency has been associated with subclinical atherosclerosis. In the general population, vitamin D deficiency has been linked with carotid and coronary atherosclerosis [32, 33]. However, high levels of serum vitamin D, occurring with overdosage, can induce hypercalcemia and hyperphosphatemia and increase the fibroblast growth factor 23 resulting in endothelium damage [34]. Although meta-analyses and most individual studies have detected an increased risk of cardiovascular events only in individuals with low 25(OH)D levels, a few studies have reported a U-shaped association, with an increased cardiovascular risk for low and high 25(OH)D levels [35, 36].

Publications in SLE patients are scarce, based on a small number of patients and have led to contradictory results. Mok et al. and Wu et al. did not find an association between subclinical atherosclerosis and serum 25(OH)D concentrations [21, 28]. These studies did not establish a relationship between serum 25(OH)D and the thickness of the IMT, carotid plaque, or the presence of coronary and aortic calcification. However, other authors such as Reynolds et al. demonstrated an inverse relationship between 25(OH)D serum levels and arterial stiffness, but not with higher IMT or carotid plaque [26]. This association persisted after being adjusted for traditional cardiovascular risk factors and body mass index. Ravenell et al. presented the first study in Afro-American SLE patients that related vitamin D deficiency to subclinical atherosclerosis determined by measuring the total carotid plaque area [37]. Sabio JM et al. found a weak inverse correlation between PWV and 25(OH)D that disappeared after adjustment for age and body mass index or systolic blood pressure [38].

In the present study, a significant correlation was found between the concentration of 25(OH)D and both the arterial stiffness, measured by PWV and IMT. These paradoxical correlations were rationalized by considering the serum calcium levels and the VitD-Ca supplements taken by the patients. A positive correlation was observed between the levels of serum calcium and both arterial stiffness and IMT. Approximately 65% of patients with arterial stiffness were receiving supplements of VitD-Ca and had higher serum calcium levels. Patients with pathological IMT also presented a significantly higher concentration of serum calcium when compared with patients with normal IMT. Thus, levels of calcium and vitamin D can have a deleterious effect on vascular calcification, and the significance of taking pharmaceutical supplements is not clear.

Studies carried out in general population have also related VitD-Ca supplements to higher arterial stiffness,

arterial calcification, and atherosclerosis [39–41]. Some authors consider the effects of vitamin D on vascular calcification as a double-edged sword, with both deficiency and excess of vitamin D causing vascular calcification [42]. Currently, the association of VitD-Ca supplements to an increased cardiovascular risk is under debate. The WHI study which followed postmenopausal women taking calcium and vitamin D did not detect any increased cardiovascular damage [43]. Treatment with moderate doses of calcium and vitamin D does not appear to result in coronary calcification [44]. However, a recent reanalysis of the WHI study has included over-the-counter supplements of calcium and vitamin D taken by patients. The authors concluded that treatment with calcium with or without vitamin D gave rise to a modest increase in the risk of cardiovascular disease [45]. These results have made the scientific community re-question an old paradigm of whether the prescription of calcium for the treatment of osteoporosis could be prejudicial for vascular health.

The present study has some limitations that must be considered. Firstly, the number of patients that could be included was limited and secondly the design of the study was based on only one determination of the serum 25(OH)D and one measurement of PWV and IMT. Finally, the study design did not include a control group of individuals without SLE but with VitD-Ca intake, as it is well described in the literature the effects of supplementation in the general population. However, it should be noted that other studies which investigated the influence of serum levels of 25(OH)D in the development of subclinical atherosclerosis in SLE patients had similar limitations.

In conclusion, SLE patients have low levels of serum vitamin D. Higher levels of vitamin D were found in patients with arterial stiffness measured by PWV and IMT. Considering all patients, our findings suggest that VitD-Ca supplements increase the serum levels of vitamin D but do not modify the serum calcium levels, however looking at the group of patients with pathological PWV, we found that most of them were on VitD-Ca supplements and had higher serum calcium levels. The significance of taking calcium and vitamin D in the pathogenesis of vascular lesion is not clear, although it may play a role in a subgroup of patients. These results could suggest a motive for concern in SLE patients taking calcium and vitamin D supplements, although prospective studies with a larger number of patients and follow-up are needed.

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Compliance with ethical standards

The study was approved by the Research Ethics Committee of the Hospital Universitario Puerta de Hierro and written informed consent was obtained from all participants.

Disclosures None.

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