25-Hydroxyivitamin D_3 levels in patients with systemic lupus erythematosus and its association with clinical parameters and laboratory tests

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ABSTRACT

Introduction: The immunoregulatory role of vitamin D has been the object of a growing number of studies in patients with systemic lupus erythematosus (SLE). **Objectives:** To determine the serum levels of 25-hydroxyvitamin D_3 [25(OH)D] in patients with SLE, and to assess the association of 25(OH)D insufficiency/deficiency with clinical parameters and laboratory tests. **Methods:** Cross-sectional, prospective study performed at the SLE Clinic, Department of Rheumatology, Hospital das Clínicas, Universidade Federal de Pernambuco with convenience sampling, including 78 patients with SLE and 64 volunteers (comparison group), matched by gender and age. **Results:** Insufficiency/deficiency of 25(OH)D was found in 45 (57.7%) patients with SLE and 25 (39%) individuals in the comparison group. The mean serum levels of 25(OH)D were 29.3 ng/mL (6.1–55.2 ng/mL) in patients with SLE and 33.12 ng/mL (15.9–63.8 ng/mL) in the comparison group, and this difference was statistically significant (P = 0.041). No statistically significant difference was observed between the mean ages of both groups. No statistically significant association was observed between 25(OH)D insufficiency/deficiency and the following: time to diagnosis; disease activity (SLEDAI ≥ 6); fatigue; use of corticosteroids and antimalarials; and anti-DNA. **Conclusions:** High prevalence of 25(OH)D insufficiency/deficiency was found in patients with SLE (57.7%), with statistically significant difference as compared with the comparison group. No association of vitamin D insufficiency/deficiency was observed with the clinical variables and laboratory tests studied. The authors emphasize the importance of determining 25(OH)D serum levels in all patients with SLE, regardless of where they live and time to disease diagnosis.

Keywords: vitamin D, autoimmune diseases, systemic lupus erythematosus.

INTRODUCTION

Systemic lupus erythematosus (SLE) is one of the most frequent autoimmune rheumatological diseases. One of its major characteristics is the presence of multiple autoantibodies, and several organs and systems are the possible targets in the disease. It affects mainly child-bearing-age women, at the proportion of 8–12:1. SLE has high morbidity rate, and death is a possible consequence.[1,2]

Although the factors contributing to the pathogeny of SLE have not yet been completely clarified, genetic mechanisms, and environmental, hormonal and immune factors are known

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to be implicated.[2] Among the environmental factors, vitamin D has been subject of an increasing number of studies in recent years, which have demonstrated its role in autoimmunity.[3–6]

Vitamin D is a steroidal hormone, whose major function consists in the regulation of calcium homeostasis, bone formation and resorption, via its interaction with parathyroid glands, kidneys and intestines.[3] Endogenous formation in skin tissues after exposure to ultraviolet B (UVB) radiation is the major source of vitamin D, which exerts its biological effect through its receptor (VDR), which is widely distributed in the body, including the immune system cells.[3] The wide distribution and expression of the VDR in most immune cells, such as monocytes, macrophages, dendritic cells, natural killer cells, and T and B lymphocytes, in addition to its effect on cell proliferation and differentiation, make vitamin D a potential candidate for immune system regulation.[7]

Patients with SLE have multiple risk factors for vitamin D deficiency. The photosensitivity characteristic of SLE determines a lower sun exposure and the use of sunscreen, which blocks UVB radiation, reducing the skin production of vitamin D. Chronic use of corticosteroids, drugs of frequent use in the treatment of patients with SLE, changes the vitamin D metabolism. In addition, severe renal impairment, which can occur in patients with lupus nephritis, can change the stage of hydroxylation of vitamin D.[8]

Some studies have linked vitamin D insufficiency/deficiency with several autoimmune diseases, such as insulin-dependent diabetes mellitus,[9] multiple sclerosis,[10] inflammatory bowel disease,[4] and SLE.[4,5,11,12] Thus, vitamin D insufficiency/deficiency is suggested to be an extrinsic factor capable of modifying the prevalence of autoimmune diseases, such as SLE,[5] and interfering with their severity.[11,12]

Studies carried out with healthy individuals in Brazil, in the cities of Recife[13] and São Paulo,[14] have shown a high prevalence of vitamin D insufficiency/deficiency. Given this context, the present study aimed to determine the serum levels of 25-hydroxyvitamin D_3 [25(OH)D] in patients with SLE seen at the Rheumatology Service,Hospital das Clínicas, Universidade Federal de Pernambuco (HC/UFPE), and we shall analyze the association of 25(OH)D insufficiency/deficiency with clinical and laboratory parameters.

PATIENTS AND METHODS

This is a cross-sectional, prospective study carried out with convenience sampling at the SLE Outpatient Clinic of the Rheumatology Service, HC/UFPE from April 2009 to March 2010. The study included 78 patients with SLE, who met at least four American College of Rheumatology (ACR) criteria for the classification of SLE,[15] and 64 volunteers, matched by gender and age, who did not have SLE and constituted the comparison group. All individuals diagnosed with other autoimmune diseases were excluded from the study.

A data collection protocol was elaborated for data recording and completed by the researchers. Information was obtained directly from the patients and their medical records. Patients were clinically assessed; then, blood samples were collected (5 mL) and centrifuged, and the sera separated and stored at -20 ºC until laboratory tests were performed. The 25(OH)D serum levels were determined by chemiluminescence (Elecsys 2010, Roche®).[16] Normal 25(OH)D serum levels were defined as values ≥ 30 ng/mL,[17] and insufficiency/deficiency as values < 30 ng/mL. Anti-DNA was assessed by *Crithidia* immunofluorescence.[18]

The association of 25(OH)D insufficiency/deficiency with the following variables was assessed: time to diagnosis, fatigue by visual analogue scale (VAS),[19] disease activity with SLEDAI,[20] corticosteroid and chloroquine use, and anti-DNA.

Data analysis comprised absolute and percentage distributions of the variables in the nominal scale and, for numerical variables, the measures of mean descriptive statistics, standard deviation, and minimum and maximum values. To assess the individual association between independent variables and presence of 25(OH)D insufficiency/deficiency, univariate analysis was performed by using Pearson chi-square test. The mean ages and 25(OH)D levels between the SLE and comparison groups were compared by use of Student *t* test for independent samples, with 95% confidence interval.

Data were entered in MS Excel software, and statistical analysis was performed with SPSS software, version 17.0. The significance level adopted was 5.0%.

This study was approved by the Ethics Committee on Research (n.1313-08) on 12/05/2008, as required by Resolution 196/96 of the Brazilian Health Board of the Ministry of Health, and Declaration of Helsinki VI, promulgated in Edinburgh, Scotland, in October 2000.

RESULTS

Insufficiency/deficiency of 25(OH)D was observed in 45 of 78 patients with SLE (57.7%) and in 25 of 64 individuals of the comparison group (39%). Table 1 shows the clinical, epidemiological and laboratory variables of patients studied.

Mean serum levels of 25(OH)D were 29.3 ng/mL (6.1–55.2 ng/mL) and 33.12 ng/mL (15.9–63.8 ng/mL) in the SLE and comparison groups, respectively. This difference was considered statistically significant. No statistically significant difference was observed between mean ages in both groups (Table 2).

No statistically significant association was observed between 25(OH)D insufficiency/deficiency and time to diagnosis, disease activity (SLEDAI ≥ 6), fatigue, use of corticosteroids and antimalarials, and anti-DNA (Table 3).

Table 1
Description of the clinical, epidemiological and laboratory variables in the SLE group

Variables	n = 78	%
Gender		
Female	76	97.4
Male	2	2.6
Time to diagnosis		
≤ 36 months	22	28.2
> 36 months	56	71.8
Fatigue	52	66.7
SLEDAI ≥ 6	20	25.6
Anti-DNA-positive	19	24.4
Antimalarial use	32	41.0
Corticosteroid use	61	78.2
Corticosteroid dose		
< 7.5 mg/day	35	44.9
7.5–30 mg/day	38	48.7
> 30 mg/day	5	6.4
Vitamin D levels		
< 30 ng/mL	45	57.7
≥ 30 ng/mL	33	42.3

Table 2
Comparison between mean ages (years) and 25(OH)D levels (ng/mL) in SLE and comparison groups

Variables	Groups				P*
	SLE (n = 78)		Comparison (n = 64)		
	Mean	SD	Mean	SD	
Age	36.9	± 10.672	38.13	± 9.704	0.625
25(OH)D levels	29.3	± 10.284	33.12	± 10.350	0.041

*Student *t* test for independent samples. CI = 95%.

Table 3
Association between 25(OH)D insufficiency/deficiency and the variables gender, time to diagnosis, fatigue, SLEDAI, anti-DNA, and use of corticosteroids and chloroquine

Variables	25(OH)D levels				Total (n = 78)	P
	< 30 ng/mL		≥ 30 ng/mL			
	n	%	n	%		
Gender						0.343**
Female	45	59.2	31	40.8	76	
Male	0	0.0	2	100.0	2	
Time to diagnosis						0.357*
≤ 36 months	15	68.2	7	31.8	22	
> 36 months	30	53.6	26	46.4	56	
Fatigue						0.808*
Yes	29	55.8	23	44.2	52	
No	16	61.5	10	38.5	26	
SLEDAI ≥ 6¥						0.971*
Yes	12	60.0	8	40.0	20	
No	29	36.9	22	43.1	51	
Anti-DNA						0.435*
Positive	9	47.4	10	52.6	19	
Negative	36	61.0	23	39.0	59	
Corticosteroid use						0.701*
Yes	34	55.7	27	44.3	61	
No	11	64.7	6	35.3	17	
Chloroquine use						0.986*
Yes	19	59.4	13	40.6	22	
No	26	56.5	20	43.5	46	

* Pearson chi-square; ** Fisher exact test.
¥ SLEDAI could not be calculated in seven patients due to lack of laboratory data.

DISCUSSION

Vitamin D is an essential nutrient for several organs and tissues, being involved in multiple biological processes, in addition to bone metabolism. In recent years, the several "roles" of vitamin D have been highlighted. A recent meta-analysis has suggested that a reduction could occur in the mortality rate of individuals undergoing supplementation.[21] In addition to its importance in general health, vitamin D has multiple immunoregulatory actions, and can be implicated in the etiopathogenesis of autoimmune diseases.[22]

Although present in several foods, its major source is still represented by its endogenous formation in cutaneous tissues after exposure to UVB radiation.[23] Depending on the latitude,

hour of the day, and individual's age, approximately 20 minutes of sun exposure can produce up to 20,000 units of vitamin D, an amount sufficient to supply the needs of an adult for approximately 25 days, and equivalent to that obtained with 200 glasses of milk.[24] However, "modern life" habits increasingly lead us to avoid the endogenous synthesis of vitamin D.

The city of Recife, in the Brazilian state of Pernambuco, where the investigation was carried out, has tropical, warm and humid climate, with mean annual temperature of 25.2 ºC. Recife is located at latitude of 8º 04' 03" South and longitude of 34º 55' 00" West, and high insolation indices throughout the year.[25] The two groups of patients in this study were from Recife and other regions of the state of Pernambuco, where there are areas with even higher insolation indices and mean annual temperatures.

Despite of the geographical location and climate characteristics, inadequate serum levels of 25(OH)D (< 30 ng/mL) were found in 57.7% of the patients with SLE and 39% of those in the comparison group. Mean serum levels were lower in the SLE group (29.3 ng/mL) as compared with those in the comparison group (33.12 ng/mL), and the difference was statistically significant.

The high prevalence of vitamin D insufficiency/deficiency found in this study, which was carried out in a high insolation region, is similar to that of other studies, which have shown inadequate 25(OH)D levels in 50%–75% of patients with SLE both in low[6,8,26] and high[27] insolation regions. The high prevalence of vitamin D insufficiency/deficiency found in our patients in this study is believed to result from the recommendations to use sunscreen and mainly to avoid sun exposure.

Although 78.2% of the patients with SLE in this study used corticosteroids, no association was found between their use and vitamin D insufficiency/deficiency. However, information bias should be considered, due to the impossibility of measuring the regularity of use of corticosteroids and their cumulative dose.

Chloroquine can inhibit 1α-hydroxylation of 25(OH)D, causing a decrease in the levels of its more active metabolite, without, however, causing alterations in 25(OH)D levels.[28] In this study, no association was found between vitamin D insufficiency/deficiency and chloroquine use, in accordance with the study by Huisman et al.[23] Those authors, however, have reported reduced serum levels of 1,25 dihydroxy vitamin D ($1,25(OH)_2D$), which were not measured in this study.

Regarding disease activity, controversy about the influence of serum levels of 25(OH)D in patients with SLE seems to exist. Similarly to the findings in other studies,[8,27,29] no association with disease activity was observed, although those studies have used different cutoff points for SLEDAI. Recently, Borba et al.[11] and Amital et al.[12] have described an association between 25(OH)D serum levels and disease activity. Considering the immunoregulatory functions of vitamin D already reported, patients with SLE and low 25(OH)D serum levels would be expected to have more severe and more often active disease. However, data in the literature are controversial. This can result from the different methodologies used, study designs, characteristics of the patients, and varied cutoff points for SLEDAI and 25(OH)D. In addition, a cutoff point from which 25(OH)D would interfere with disease activity is not known. Thus, more studies with larger populations are required to define the actual role of vitamin D in the activity of disease in patients with SLE and to define from which 25(OH)D serum levels the disease activity would be influenced.

Vitamin D deficiency has been identified as the cause of muscular weakness and myalgia in elderly patients,[30,31] and an association of low 25(OH)D serum levels with fatigue in patients with SLE has been reported.[8] Although fatigue was present in 66.6% of patients with SLE, no association with 25(OH)D insufficiency/deficiency has been identified. This study did not assess the association between fatigue in patients with SLE and disease activity, use of drugs, and comorbidities. These factors could justify the prevalence of fatigue observed in patients with SLEs in this study.

In this study, no association was observed between vitamin D insufficiency/deficiency and the time to diagnosis. Serum levels of 25(OH)D lower than 30 ng/mL were observed in 15 of 22 patients (68.2%) diagnosed with SLE within less than three years. This suggests that in the first years of diagnosis, vitamin D insufficiency/deficiency can occur. Thus, measuring 25(OH)D should be recommended at the time SLE is diagnosed, because high prevalence of vitamin D insufficiency/deficiency has also been reported in previously healthy individuals,[31,32] as well as in the first years of the disease.

The association between vitamin D insufficiency/deficiency and anti-DNA in patients with SLE is controversial. This study found no association, in accordance with that by Toloza et al.,[6] and contrary to that by Carvalho et al.,[33] who reported an association between 25(OH)D serum levels and presence of anti-DNA. More studies are required to assess the association of the autoantibody profile and 25(OH)D serum levels.

Vitamin D insufficiency/deficiency seems not to be an exclusivity-of patients with SLE, although such patients usually have lower mean serum levels than the healthy population, as shown in this study. Inadequate vitamin D levels also occur in healthy children, young adults, elderly, and hospitalized patients.[17] This relationship has also been verified in a recent meta-analysis[31] with healthy Caucasian individuals.

In the present study, the percentage of vitamin D insufficiency/deficiency in the comparison group (25/64, 39%), was similar to that of European studies (40%–80% prevalence) described in a recent review.[32] That high prevalence reported for healthy individuals is likely to result from "modern life" activities, which make us avoid sun exposure, and, consequently, reduce vitamin D synthesis.

The major limitation of this study was the impossibility to assess the cumulative dose of corticosteroids in patients using or who had used those drugs and the regularity of sunscreen use. The influence of ethnicity was not assessed in this study, because that variable is difficult to define in the Brazilian population. Another difficulty was the comparison of the results in this study with those of other studies, due to the different levels of insolation of the regions assessed. In addition, other aspects, such as methodology, study design, characteristics of patients, and varied cutoff points for SLEDAI, made comparisons difficult.

In conclusion, according to this study's findings, 57.7% of patients with SLE showed low 25(OH)D serum levels, which were significantly lower than those in the comparison group. No association between vitamin D insufficiency/deficiency and the following was observed: time to diagnosis; disease activity; fatigue; anti-DNA; and use of corticosteroids and chloroquine.

Thus, considering the multiple "roles" of vitamin D, we emphasize the importance of determining 25(OH)D serum levels in all patients with SLE, regardless of where they live and the time to disease diagnosis.

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