ORIGINAL ARTICLE

VITAMIN D LEVELS ARE ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY

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Abstract

The objective of the present paper was to establish the correlation of vitamin D (VD) with various systemic lupus erythematosus (SLE) specific variables. The study comprised 65 SLE patients that prospectively underwent clinical and laboratory (including VD status) evaluation. Our results showed that abnormal VD levels were highly prevalent among SLE patients; low VD levels and VD deficiency are associated with higher disease activity, higher damage and glucocorticoid treatment; VD deficiency was an independent risk factor for flare and high disease activity, while VD supplementation was a protective factor for flare status. Lymphopenia was associated with lower VD levels, VD insufficiency was more prevalent among SLE patients with cutaneous involvement and normal VD levels were more prevalent among SLE patients with neurologic involvement. Abnormal VD levels were not associated with SLE serology markers. In conclusion hypovitaminosis D can be considered a SLE disease manifestation and routine VD testing and supplementation in SLE could be beneficial.

Rezumat

Obiectivul prezentului studiu a fost analiza relației dintre vitamina D (VD) și variabilele specifice lupusului eritematos sistemic (LES). Au fost incluși în studiu 65 de pacienți, internați cu LES, și s-a efectuat în mod prospectiv o evaluare clinică și paraclinică (inclusiv determinarea cantitativă VD). Rezultatele au arătat titruri anormale de VD foarte prevalente în rândul pacienților cu LES; titruri scăzute de VD și deficiența de VD s-au asociat cu o activate mai intensă a bolii, afectare organică mai importantă și tratament cu glucocorticoizi; deficiența de VD s-a dovedit un factor de risc independent pentru activitatea înaltă a bolii, iar suplimentarea cu VD, un factor protector pentru reactivarea bolii. Limfopenia s-a asociat cu hipovitaminoza D, insuficiența de VD a fost mai frecventă la pacienții cu afectare cutanată iar titrurile normale de VD au fost mai frecvente la pacienții cu afectare neurologică. Titrurile anormale de VD nu s-au asociat cu serologica specifică LES. În concluzie, hipovitaminoza D ar putea fi considerată o manifestare a LES, iar testarea și suplimentarea de rutină a VD în LES ar putea fi indicată.

Keywords: vitamin D, systemic lupus erythematosus, disease activity

Introduction

Vitamin D is a steroid hormone that has a specific role in mineral metabolism, skeletal function but also in the immune system [1]. It has an important effect on growth, apoptosis, proliferation and proper function of the immune cells [1-3]. This is due to immune cells, like monocytes, macrophages, dendritic cells, activated T and B cells and also cells of the innate immunity, that have vitamin D receptors [4]. It is recognized that vitamin D deficiency is very frequent and can be associated with disease states [5]. Epidemiological data indicate a significant association between vitamin D deficiency and autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, multiple sclerosis [3-4] The involvement of vitamin D in autoimmunity is also suggested by a recent study which found that antinuclear antibody (ANA)-positive healthy controls are significantly more likely to be deficient in vitamin D than ANA-negative healthy controls [6], establishing a connection between vitamin D and autoantibody production.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, currently incompletely understood, in which high autoantibody production is one of the most important pathogenic features. The link between vitamin D and SLE was first described in 1995 [7]. Hypovitaminosis D is highly prevalent in SLE patients from different genetic backgrounds [8-16] for the following reasons: lifestyle (avoidance of sunshine exposure and use of sunscreen), chronic kidney disease and the use of different medications that alter the metabolism of vitamin D or down-regulate the functions of the

vitamin D receptors. The physiologic and clinical consequences of vitamin D deficiency in SLE are not entirely known [17], but recent studies found a possible association of low vitamin D status and disease activity in SLE [6, 8, 10, 14, 18, 19]. This suggests that supplementation with vitamin D could add benefits beyond bone health for these patients, acting probably as a potential disease modifying drug for SLE. In this context, the study aims to establish the relation of vitamin D with various SLE-specific variables (disease activity, specific organ involvement, serology and treatment).

Materials and Methods

Patients

The study was designed to prospectively include patients consecutively admitted randomly in the Department of Internal Medicine and Rheumatology of "Sfânta Maria" Clinical Hospital (Bucharest, Romania) between October 2011 and April 2012. This study frame was selected in the hope of observing a relative uniformization of patient exposure to natural UV light. The inclusion criteria were age above 18 years and fulfilment of the 1997 American College of Rheumatology (ACR) criteria for SLE [20]. The following exclusion criteria were applied: SLE overlap with other chronic autoimmune inflammatory rheumatic diseases, cancer and active chronic or acute infections, pregnancy. All the patients gave a written informed consent and the study was approved by the local ethics committee.

Variables

Each patient underwent in the same day a clinical interview, a physical examination (both done by the same senior rheumatologist) and peripheral venous blood sample collection. The clinical interview and the medical documents served as information sources for demographic variables (age, gender, dwelling, smoking and menopause status), and along with the physical examination, for SLE-specific variables (disease duration, type of clinical involvement and treatment regimens). The recorded SLE clinical manifestations were those needed to assess disease activity using the modified Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [21]. Using the SLEDAI, disease states were defined as follows [22]: inactive (SLEDAI = 0), low disease activity (LDA, SLEDAI = 1-5), moderate disease activity (MDA, SLEDAI = 6-10) and high disease activity (HDA, SLEDAI $\geq$ 11). Disease flare was defined using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) SLEDAI Flare Index [23]. The extent of damage was assessed using the Systemic Lupus International Collaborating Clinics (SLICC)-ACR Damage Index (SDI) [24].

Serum vitamin D was measured by enzyme-linked immunosorbent assays (ELISA) using the 25-hydroxyvitamin D DIAsource kit, in accordance with the manufacturer's recommendations. Using the titers of serum 25-hydroxyvitamin D (calcidiol), each patient was classified as [2]: normal ($\geq$ 30 ng/mL), vitamin D insufficiency (21-30 ng/mL); vitamin D deficiency (< 20 ng/mL). Anti-double stranded deoxyribonucleic acid (dsDNA) antibodies, anti-Sm antibodies and C-reactive protein (CRP) were assessed by ELISA using commercially available kits, and were considered positive/high at titers above 50 IU/mL, 20 IU/mL and 5 mg/L respectively. Complement fractions (C3 and C4) were detected by immune-electrophoresis and were considered high if C3 > 90 mg/dL and respectively if C4 > 14 mg/dL. Lymphopenia was defined by a lymphocyte count bellow 1000/μL.

Statistics

The normal distribution of data was assessed using descriptive statistics, normality and stem-and-leaf plots, and the Lilliefors corrected Kolmogorov-Smirnov test. All the scale variables were non-normally distributed, therefore they were reported as "median (interquartile range)", their correlations were assessed using bivariate Spearman coefficients and their differences among subgroups were assessed with Mann Whitney and Kruskal Wallis tests (for subgroups with two or more categories respectively). Nominal variables were expressed as absolute and percent frequency and their associations were assessed using χ^2 tests (or Fisher tests where appropriate). Post-hoc analyses were performed in order to determine which categories of multi-level nominal variables produced significant Kruskal Wallis and χ^2 tests: Dunn-Bonferroni analysis and respectively the study of adjusted standardized residuals. Binary logistic regression models were computed in order to predict disease activity states ("flare", coded "0" for "no" and "1" for "yes", and "SLEDAI category", coded "0" for "inactive and LDA" and "1" for "MDA and HDA") using vitamin D independent variables and covariates (age, gender, disease duration, glucocorticoids). The statistical tests were considered significant if $p < 0.05$. All the tests were performed using IBM SPSS Statistics version 22.0 for Windows (Armonk, NY, IBM Corp.). Figure 4 was generated using GraphPad Prism version 6.04 for Windows (GraphPad Software, La Jolla California USA).

Results and Discussion

General characteristics

The study included 65 predominantly urban-dwelling Caucasian SLE patients with a median age of 44 (29) years (Table I). The sample included only 4 men (6.2%) with SLE. Half of the female SLE patients were at menopause.

Table I

General characteristics of the sample (n = 65)

demographics	age (years)	44 (29)
	women (n)	61 (93.8%)
	urban (n)	47 (72.3%)
	smoking (n)	13 (20.0%)
	menopause (n)	32 (49.2%)[a]
	early menopause (n)[b]	4 (6.2%)[c]
vitamin D	serum calcidiol (ng/mL)	21 (11)
	vitamin D supplement (n)	50 (76.9%)
	vitamin D 1000 IU/day (n)	36 (55.4%)
	vitamin D 2000 IU/day (n)	14 (21.5%)
SLE	disease duration (years)	5 (9)
	anti-Sm antibodies (n)	16 (24.6%)
	low serum C3 (n)	20 (30.8%)
	low serum C4 (n)	11 (16.9%)
	high CRP[d] (n)	16 (24.6%)
	SLEDAI	6 (6)
	flare (n)	30 (46.2%)
	glucocorticoids (mg/day)[e]	8 (12)

Notes: [a] percentage from the total number of women; [b] early menopause defined at an age of onset of < 40 years; [c] percentage from the total number of women in menopause; [d] associated with serositis and arthritis;[e] calculated for the 50 cases on glucocorticoid treatment; scale variables are reported as "median (interquartile range)"; nominal variables are reported as "observed frequency (percentage from sample)"; normal ranges: C3 mg/dL; C4 mg/dL; CRP mg/L; calcidiol ≥ 30 ng/mL.

Abbreviations: C3/4 – serum complement fraction 3/4; CRP – C-reactive protein; IU – international units; SLEDAI – Systemic Lupus Erythematosus (SLE) Disease Activity Index.

SLE characteristics

The median SLE disease duration of the studied population was 5 (9) years. Exactly 80% of the patients had positive anti-dsDNA antibodies (Figure 1A). Almost half of the patients (30 cases – 46.2%) were having a disease flare at the time of inclusion (Table I). The most common clinical manifestations were cutaneous, articular and hematologic involvement (Figure 2A). Most of the patients had either MDA (41.5%) or LDA (52.3%) according to SLEDAI (Figure 2B). Only 15.4% of the patients had extensive tissue damage (SDI ≥ 2 events) caused by the disease (Figure 2C). More than three quarters were receiving glucocorticoids and hydroxychloroquine, while half of them were receiving other immuno-suppressant drugs (Figure 2B).

Vitamin D

Only 10.8% of our patients had normal vitamin D levels (Figure 1D), in spite of the fact that 76.9% of them were receiving vitamin D supplements and those who did had significantly higher vitamin D levels (Table I).

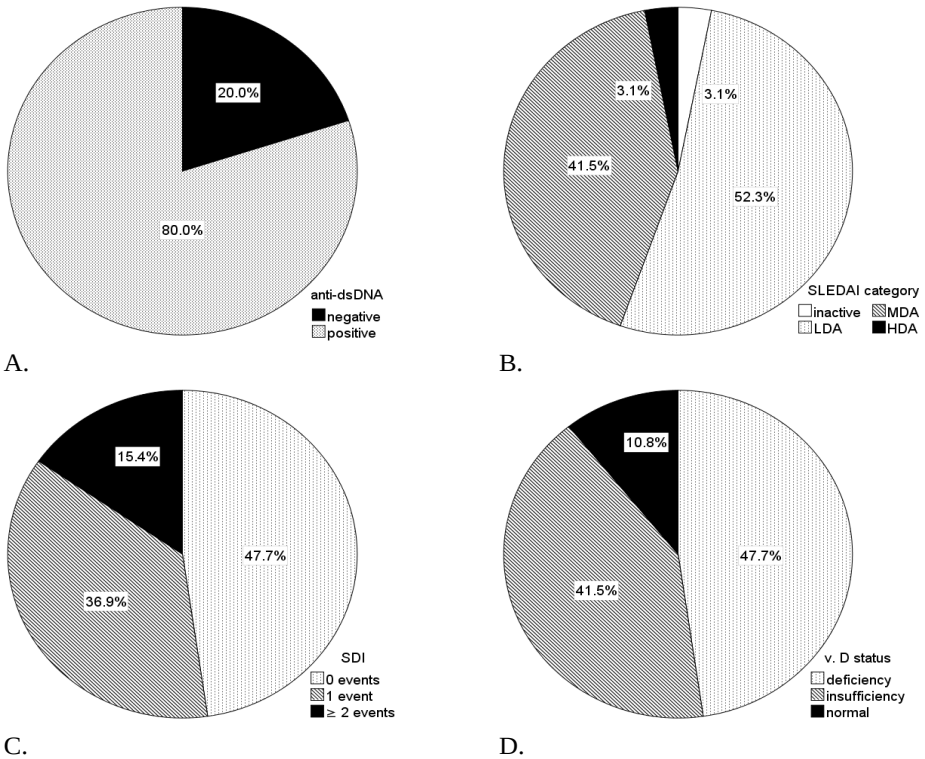

Figure 1.

Characteristics of the SLE sample (n = 65): A. Anti-dsDNA antibodies; B. SLEDAI categories; C. SDI distribution; D. Vitamin D status.

Abbreviations: dsDNA – double stranded deoxyribonucleic acid; L/M/HDA – low/moderate/high disease activity; SDI – SLICC (Systemic Lupus Collaborating Clinics) ACR (American College of Rheumatology) Damage Index; SLEDAI – Systemic Lupus Erythematosus (SLE) Disease Activity Index.

Compared to each respective subgroup, the patients with disease flare, high CRP, lymphopenia, high SDI and high SLEDAI-defined disease activity had significantly lower titers of vitamin D (Figure 3, Table II). Unexpectedly, patients with neurologic involvement had significantly higher vitamin D levels and significantly higher prevalence of normal vitamin D levels compared to patients without neurologic involvement (Table II). Patients with vitamin D deficiency had a significantly higher SLEDAI, dose of daily glucocorticoids, prevalence of low C3, disease flare and SLEDAI-defined high disease activity (Table II). Patients with vitamin D insufficiency had a significantly higher prevalence of cutaneous involvement, while patients with normal vitamin D levels had a lower prevalence of cutaneous involvement and low C3 (Table II). Binary logistic regression analysis results are showed Figure 4.

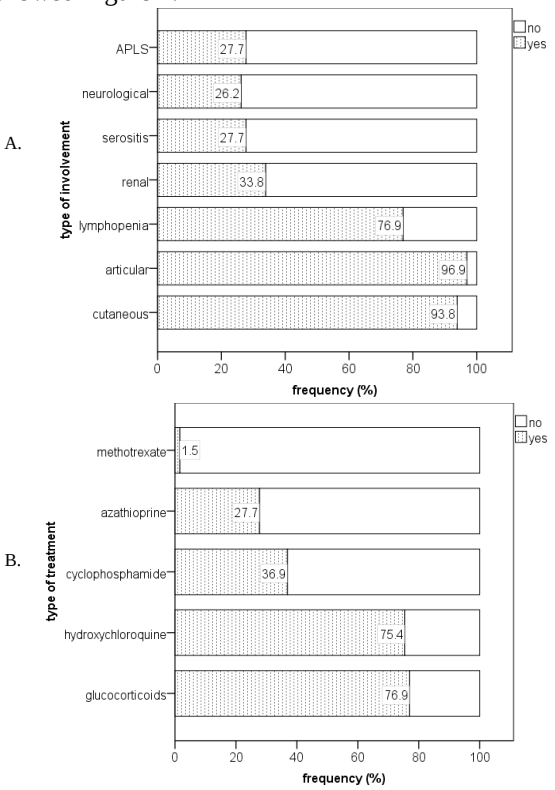

Figure 2.

Types of clinical involvement (A) and types of treatment (B) in the studied sample (n = 65). The reported values represent percentages of the total sample.

Abbreviation: APLS – anti-phospholipid syndrome.

The study allowed observations which fulfilled its objective: abnormal vitamin D levels are highly prevalent among SLE patients; low vitamin D levels and vitamin D deficiency are associated with higher disease activity, higher damage and gluco-corticoid treatment; vitamin D deficiency is a significant and independent risk factor for flare status and high disease activity, while vitamin D supplementation is a protective factor for flare status; among specific disease manifestations, lymphopenia is associated with lower vitamin D levels [9], vitamin D insufficiency is more prevalent among SLE patients with cutaneous involvement and normal vitamin D levels are more prevalent among SLE patients with neurologic involvement; abnormal vitamin D levels and states were not associated with SLE serology markers (anti-dsDNA [10] and anti-Sm antibodies) nor with the antiphospholipid syndrome [14]. All these results are statistically significant, but their relevance should be discussed.

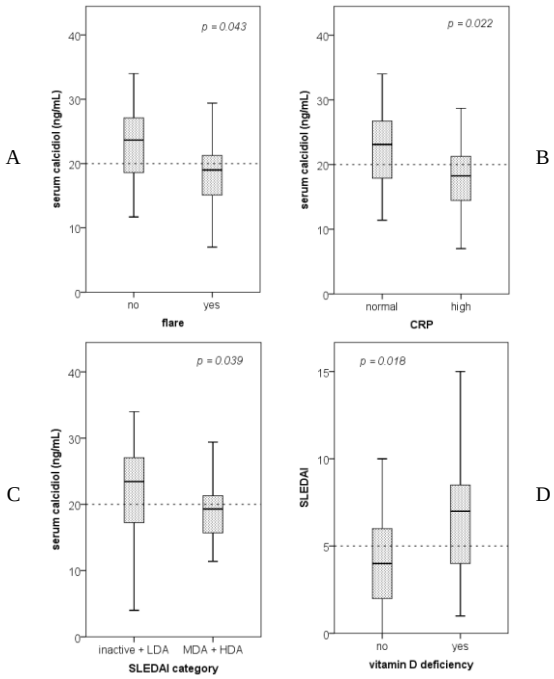

Figure 3.

Significant differences of vitamin D variables in SLE subgroups. A-C: patients with disease flare, high CRP and high disease activity (defined by SLEDAI categories) have significantly lower titers of calcidiol. D: patients with vitamin D deficiency have a significantly higher SLEDAI score. p values represent the significance of Mann Whitney tests.

Abbreviations: CRP – C-reactive protein; L/M/HDA – low/ moderate/high disease activity; SLEDAI – Systemic Lupus Erythematosus Disease Activity Index.

In order to offer a relevant interpretation of the results there are two aspects we need to discuss: the study group and the literature. Our sample had long-standing disease, 15% had extensive SLE-related organ damage, half were flaring at the time of inclusion and three quarters were receiving glucocorticoids. It would be interesting to observe these results in a sample with early SLE, since there are reports that SLE patients have a higher prevalence of vitamin D deficiency ever since their diagnosis [10] and since disease duration may be

directly proportional with the prevalence of abnormal vitamin D levels (an hypothesis which was not confirmed by our data). Also, a study group in which the proportion of patients not receiving glucocorticoids would be greater is of interest, especially because there are conflicting reports that one hand the cumulative dose of glucocorticoids significantly influences vitamin D levels in SLE patients [19], and on the other hand that vitamin D levels are not associated with glucocorticoids [13] (a discrepancy which might have a connection with the geographical location of the observations, namely Canada and Spain). Our observation of the high prevalence of abnormal vitamin D levels in SLE patients is confirmed by other studies which reported similar frequencies [12, 19]. The studies investigating the link between vitamin D and SLE activity/damage have mixt results: our observations confirm most reports of a significant association between vitamin D levels and disease activity/ damage [8, 12, 14, 18], but there are studies in which this association was not significant [13, 15, 16].

Table II

Significant differences and associations of vitamin D variables in SLE subgroups

lymphopenia	no (n = 15)	yes (n = 50)		p
calcidiol (ng/mL)	21.5 (10.1)	20.3 (11.2)		0.048
neurologic involvement	no (n = 48)	yes (n = 17)		
calcidiol (ng/mL)	19.6 (9.9)	24.7 (9.4)		0.048
SDI	0 (n = 31)	1 (n = 24)	≥ 2 (n = 10)	
calcidiol (ng/mL)	23.4 (8.4)[a]	18.4 (7.1)[a]	25.7 (11.9)	0.047
vitamin D deficiency	32.3% (10)[a]	70.8% (17)[a]	40.0% (4)	0.015
D supplement	no (n = 15)	yes (n = 50)		
age at diagnosis (years)	27 (14)	36 (18)		0.026
D supplement (IU/day)	0 (n = 15)	1000 (n = 36)	2000 (n = 14)	
calcidiol (ng/mL)	18.3 (6.7)[a]	23.4 (7.9)[a]	18.4 (11.2)	0.048
age at diagnosis (years)	27 (14)[a]	37 (17)[a]	34 (16)	0.042
vitamin D deficiency	no (n = 34)	yes (n = 31)		
GC (mg/day)	4 (7)	8 (12)		0.040
low C3	20.6% (7)	41.9% (13)		0.043
with flare	32.3% (11)	61.3% (19)		0.019
vitamin D status	deficiency (n = 31)	insufficiency (n = 27)	normal (n = 7)	
SLEDAI	7 (5)[a,b]	4 (4)[a]	4 (8)[b]	0.041
normal C3	58.1% (18)[a]	74.1% (20)	100% (7)[a]	0.043
with flare	61.3% (19)[a]	33.3% (9)[a]	28.6% (2)	0.043
cutaneous involvement	93.5% (29)	100% (27)[a]	71.4% (5)[a]	0.020
SLEDAI category	inactive+LDA (n = 36)	MDA+HDA (n = 29)		
GC (mg/day)	4 (4)	8 (12)		0.001
with GC	66.7% (24)	89.7% (26)		0.029
vitamin D deficiency	36.1% (13)	62.1% (18)		0.037
normal vitamin D	no (n = 58)	yes (n = 7)		
low C3	34.5% (20)	0.0% (0)		0.042
neurologic involvement	22.4% (13)	57.2% (4)		0.048
cutaneous involvement	96.6% (56)	71.4% (5)		0.009

Notes: scale variables are reported as "median (interquartile range)"; - nominal variables are reported as "subgroup percentage (observed count)"; p values represent the significance of Mann Whitney tests (for nominal subgroup variables with two categories, for example "flare"), Kruskal Wallis tests (for nominal subgroup variables with three categories, for example "vitamin D status"), χ^2 (or Fisher tests for two nominal variables; post-hoc analysis for subgroup variables with three categories identified the subgroups which differed significantly and which are marked with "a" and "b".

Abbreviations: C3 – serum complement fraction 3; CRP – C-reactive protein; GC – glucocorticoids; IU – international units; L/M/HDA – low/ moderate/high disease activity; SDI – SLICC (Systemic Lupus Collaborating Clinics) ACR (American College of Rheumatology) Damage Index; SLEDAI – Systemic Lupus Erythematosus Disease Activity Index.

Since all of these studies varied greatly in terms of design, geographical location and patient characteristics, there are many confusing factors (some of which are unknown) that preclude a definite statement of whether SLE activity/damage and vitamin D levels/states are significantly associated. Large prospective multinational cohorts would offer convincing evidence in this matter. In either case, presuming our results to be correct, the high prevalence of abnormal vitamin D levels (89.2% had vitamin D insufficiency or deficiency, a frequency paralleled only by arthralgia/arthritis and cutaneous involvement in SLE [25]) and the significant and independent association of vitamin D status with disease activity/damage suggest that hypovitaminosis D is actually a SLE disease manifestation. The hypothesis that low hypo-vitaminosis D is a causal consequence of SLE is supported by a number of theoretical and observational arguments: vitamin D levels are

lower in SLE patient compared to healthy controls [26], but vitamin D intake is not associated with the risk of developing SLE [27]; the observation is coherent, consistent and it displays a temporal relationship and gradient effect. However, the low strength of the associations, its low specificity, the high prevalence of glucocorticoid treatment and low sun exposure are valid contra-arguments. If hypovitaminosis D is indeed a biological SLE manifestation, it should be routinely screened in clinical practice. Routine measurement and supplementation of vitamin D in SLE may be indicated based on our observations and those reported in the literature: the high prevalence of hypovitaminosis D in SLE, its significant and independent association with disease activity/damage and the fact that vitamin D supplementation was a significant protective factor for flare status (Petri *et al.* showed that a therapeutically-induced increase of serum calcidiol lowers the risk of high disease activity [28]). The potential clinical (disease activity, osteoporosis etc.[29]) and biological benefits [30] and the relative low risk of side effects indicate that routine vitamin D supplementation in SLE can be part of the good clinical practice.

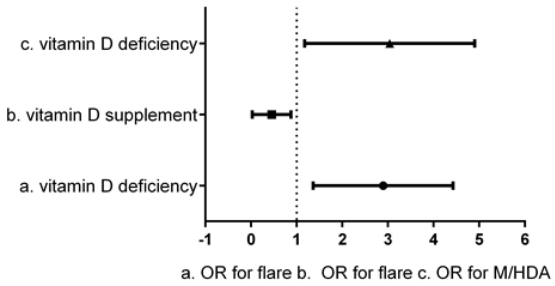

Figure 4.

Vitamin D adjusted odds ratio (OR) for flare (a. and b.) and for moderate and high disease activity (M/HDA) (c.). Model a: prediction rate 64.6%, $\chi^2 = 5.5$, $p = 0.019$, $R^2 = 0.109$. Model b: prediction rate 61.5%, $\chi^2 = 3.3$, $p = 0.048$, $R^2 = 0.038$. Model c: prediction rate 63.1%, $\chi^2 = 4.4$, $p = 0.036$, $R^2 = 0.087$.

The study has several limitations which could have influenced the results and their interpretation: the relative small sample size, the absence of a control group, the lack of information regarding cumulative doses of glucocorticoids, photosensitivity and photo-protection.

Conclusions

Hypovitaminosis D is highly prevalent among SLE patients and it is a significant predictor of disease activity, while vitamin D supplementation is a protective factor for SLE flare status. Therefore hypovitaminosis D can be considered a SLE disease manifestation. Routine vitamin D testing and supplementation in SLE could be beneficial.

References

1. Arnson Y., Amital H., Shoenfeld Y., Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Annals of the rheumatic diseases*, 2007; 66(9): 1137-1142.
2. Cutolo M., Further emergent evidence for the vitamin D endocrine system involvement in autoimmune rheumatic disease risk and prognosis. *Annals of the rheumatic diseases*, 2013; 72(4): 473-475.
3. Cutolo M., Vitamin D or hormone D deficiency in autoimmune rheumatic diseases, including undifferentiated connective tissue disease. *Arthritis research & therapy*, 2008; 10(6): 123.
4. Kongsbak M., Levring T.B., Geisler C., von Essen M.R., The vitamin d receptor and T cell function. *Frontiers in immunology*, 2013; 4: 148.
5. Holick M.F., Vitamin D deficiency. *The New England journal of medicine*, 2007; 357(3): 266-281.
6. Ritterhouse L.L., Crowe S.R., Niewold T.B., Kamen D.L., Macwana S.R., Roberts V.C., Dedeke A.B., Harley J.B., Scofield R.H., Guthridge J.M., Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. *Annals of the rheumatic diseases*, 2011; 70(9): 1569-1574.
7. Muller K., Kriegbaum N.J., Baslund B., Sorensen O.H., Thymann M., Bentzen K., Vitamin D3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D3 in patients with systemic lupus erythematosus. *Clinical rheumatology*, 1995; 14(4): 397-400.
8. Amital H., Szekanecz Z., Szucs G., Danko K., Nagy E., Csepany T., Kiss E., Rovensky J., Tuchynova A., Kozakova D., Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D? *Annals of the rheumatic diseases*, 2010; 69(6): 1155-1157.
9. Bogaczewicz J., Sysa-Jedrzejowska A., Arkuszewska C., Zabek J., Kontny E., McCauliffe D., Wozniacka A., Vitamin D status in systemic lupus erythematosus patients and its association with selected clinical and laboratory parameters. *Lupus*, 2012, 21(5): 477-484.
10. Bonakdar Z.S., Jahanshahifar L., Jahanshahifar F., Gholamrezaei A., Vitamin D deficiency and its association with disease activity in new cases of systemic lupus erythematosus. *Lupus*, 2011; 20(11): 1155-1160.
11. Borba V.Z., Vieira J.G., Kasamatsu T., Radominski S.C., Sato E.I., Lazaretti-Castro M., Vitamin D deficiency in patients with active systemic lupus erythematosus. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, 2009; **20**(3): 427-433.
12. Hamza R.T., Awwad K.S., Ali M.K., Hamed A.I., Reduced serum concentrations of 25-hydroxy vitamin D in Egyptian patients with systemic lupus

erythematosus: relation to disease activity. *Medical science monitor: international medical journal of experimental and clinical research*, 2011; 17(12): CR711-718.
13. Lopez-Robles C., Rios-Fernandez R., Callejas-Rubio J.L., Ortego-Centeno N., Vitamin D deficiency in a cohort of patients with systemic lupus erythematous from the South of Spain. *Lupus*, 2011, 20(3): 330-331.
14. Mok C.C., Birmingham D.J., Leung H.W., Hebert L.A., Song H., Rovin B.H., Vitamin D levels in Chinese patients with systemic lupus erythematosus: relationship with disease activity, vascular risk factors and atherosclerosis. *Rheumatology*, 2012; 51(4): 644-652.
15. Ruiz-Irastorza G., Egurbide M.V., Olivares N., Martinez-Berriotxoa A., Aguirre C., Vitamin D deficiency in systemic lupus erythematosus: prevalence, predictors and clinical consequences. *Rheumatology*, 2008; 47(6): 920-923.
16. Ciorba D., Tataru A., Avram A., Almasi A., Moldovan M., Influence of environmental chloroform concentrations on biophysics skin parameters. Farmacia, 2015; 63(2): 313-317.
17. Sakthiswary R., Raymond A.A., The clinical significance of vitamin D in systemic lupus erythematosus: a systematic review. *PloS one*, 2013, 8(1): e55275.
18. Yap K.S., Northcott M., Hoi A.B., Morand E.F., Nikpour M., Association of low vitamin D with high disease activity in an Australian systemic lupus erythematosus cohort. *Lupus science & medicine*, 2015; 2(1): e000064.
19. Toloza S.M., Cole D.E., Gladman D.D., Ibanez D., Urowitz M.B., Vitamin D insufficiency in a large female SLE cohort. *Lupus*, 2010; 19(1): 13-19.
20. Hochberg M.C., Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis and rheumatism*, 1997; 40(9): 1725.
21. Gladman D.D., Ibanez D., Urowitz M.B., Systemic lupus erythematosus disease activity index 2000. *The Journal of rheumatology*, 2002; 29(2): 288-291.
22. Petri M., Genovese M., Engle E., Hochberg M., Definition, incidence, and clinical description of flare in systemic lupus erythematosus. A prospective cohort study. *Arthritis and rheumatism*, 1991; 34(8): 937-944.
23. Buyon J.P., Petri M.A., Kim M.Y., Kalunian K.C., Grossman J., Hahn B.H., Merrill J.T., Sammaritano L., Lockshin M., Alarcon G.S., The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. *Annals of internal medicine*, 2005; 142(12 Pt 1): 953-962.
24. Gladman D., Ginzler E., Goldsmith C., Fortin P., Liang M., Urowitz M., Bacon P., Bombardieri S., Hanly J., Hay E., The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis and rheumatism*, 1996; 39(3): 363-369.
25. Căruntu C., Negrei C., Ghiţă M.A., Căruntu A., Bădărău A.I., Buraga I., Boda D., Albu A., Brănişteanu D., **C**apsaicin, a hot topic in skin pharmacology and physiology. *Farmacia*, 2015; 63(4): 487-491.
26. Kamen D.L., Cooper G.S., Bouali H., Shaftman S.R., Hollis B.W., Gilkeson G.S., Vitamin D deficiency in systemic lupus erythematosus. *Autoimmunity reviews*, 2006, 5(2): 114-117.
27. Costenbader K.H., Feskanich D., Holmes M., Karlson E.W., Benito-Garcia E., Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Annals of the rheumatic diseases*, 2008; 67(4): 530-535.
28. Petri M., Bello K.J., Fang H., Magder L.S., Vitamin D in systemic lupus erythematosus: modest association with disease activity and the urine protein-to-creatinine ratio. *Arthritis and rheumatism*, 2013; 65(7): 1865-1871.
29. Munoz-Ortego J., Torrente-Segarra V., Prieto-Alhambra D., Salman-Monte T.C., Carbonell-Abello J., Prevalence and predictors of vitamin D deficiency in non-supplemented women with systemic lupus erythematosus in the Mediterranean region: a cohort study. *Scandinavian journal of rheumatology*, 2012; 41(6): 472-475.
30. Pons-Estel B.A., Catoggio L.J., Cardiel M.H., Soriano E.R., Gentiletti S., Villa A.R., Abadi I., Caeiro F., Alvarellos A., Alarcon-Segovia D., The GLADEL multinational Latin American prospective inception cohort of 1,214 patients with systemic lupus erythematosus: ethnic and disease heterogeneity among "Hispanics". *Medicine*, 2004, 83(1): 1-17.