

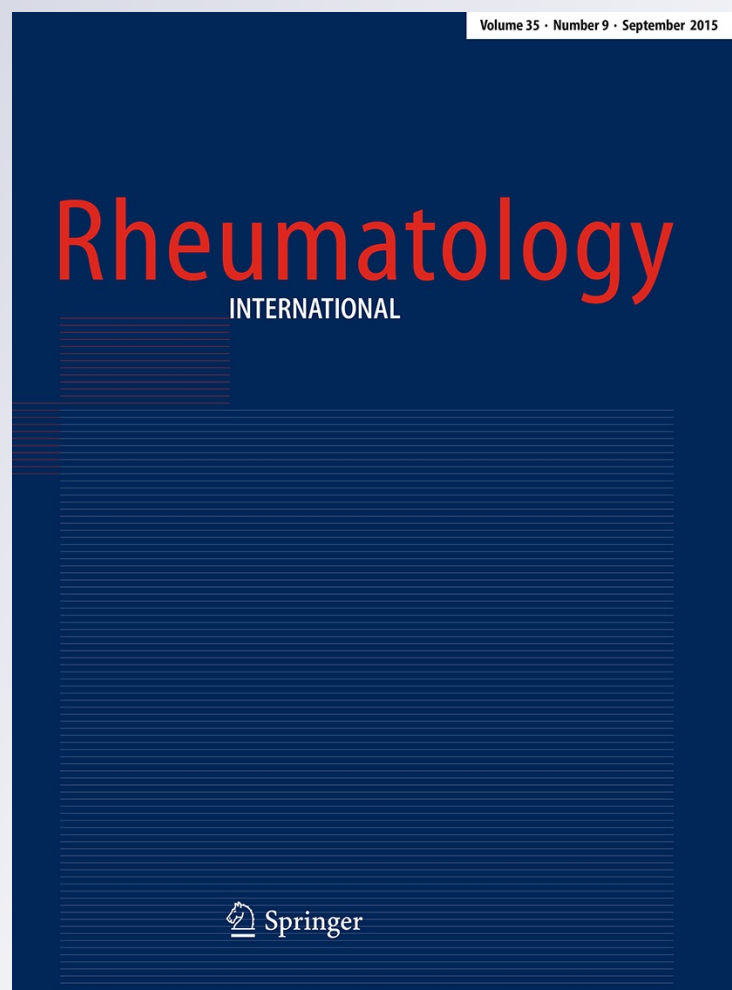
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Vitamin D status in Egyptian patients with juvenile-onset systemic lupus erythematosus

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Abstract There are scanty data on the prevalence of vitamin D deficiency and its relation to disease activity among patients with juvenile-onset systemic lupus erythematosus (JoSLE) in the Middle East and North Africa, an area known to be endemic for vitamin D deficiency and insufficiency. The aim of this study was, therefore, to study vitamin D status and its relation to disease activity and parameters in Egyptian patients with JoSLE. Serum levels of 25(OH) D3 in 70 JoSLE patients were compared to 40 age-, sex-, and body mass index-matched healthy controls. The 25(OH) D3 was determined by enzyme-linked immunosorbent assay. Information regarding the medical history, clinical symptoms, and signs was registered at the time of serum sampling. Disease activity of SLE was evaluated according to the SLEDAI score. The mean level of serum 25(OH) D3 was 12 ± 3.7 in JoSLE patients compared to 21 ± 3.5 ng/mL in normal controls ($p < 0.001$). Forty percent (28/70) of the JoSLE patients has severe 25(OH) D3 deficiency (≤ 10 ng/mL), and 60 % of the JoSLE patients has 25(OH) D3 insufficiency (≤ 30 ng/mL). None of the JoSLE patients has 25(OH) D3 level >30 ng/mL. There was no significant correlation between serum levels of 25(OH) D3 and the demographic data, medication used, and some laboratory data of patients with JoSLE. Disease

activity score in our patients was insignificantly correlated with serum levels of 25(OH) D3. In spite of vitamin D supplementation in Egyptian JoSLE patients and the presence of vitamin D insufficiency in the control group, there are still significantly lower levels of vitamin D in JoSLE compared to normal controls.

Keywords Vitamin D insufficiency · Severe vitamin D deficiency · Juvenile-onset SLE (JoSLE) · Disease activity

Introduction

Vitamin D is a steroid hormone that possesses many regulatory effects on the immune system. These include inhibitory effects on T and B lymphocytes and dendritic cells. CD4 T lymphocytes express the VDR (vitamin D receptors), and these receptors are upregulated fivefold upon activation [1]. Vitamin D ameliorates T cell receptor-induced T cell proliferation [2, 3] and promotes generation of regulatory T cells, and it specifically appears to inhibit Th1 cell proliferation [4, 5] with a shift toward a Th2 response [6, 7]. Vitamin D has a direct effect on B cells, decreasing B cell differentiation, and inhibits immunoglobulin production [8]. Physiologic levels of 1, 25D inhibit the maturation of dendritic cells with inhibition of activation markers such as major histocompatibility complex (MHC) class II, and many of the costimulatory molecules such as CD40, CD80, and CD86 [9]. Furthermore, 1, 25D downregulates the dendritic cell production of IL-12 and augments IL-10 [10]. These effects on the immune system have raised the issue of role of vitamin D in autoimmune diseases such as systemic lupus erythematosus (SLE).

The main source of vitamin D is synthesis by the human skin exposed to UVB radiation. Dietary intake contributes

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only to 20 % of the body's requirements of vitamin D [11]. Despite ample sunshine, vitamin D deficiency is very common in the Middle East and North African countries. Low vitamin D concentrations were reported among ethnic Saudi Arabians [12–16], Oman [17–19], United Arab Emirates [20–22], and Qatar [23, 24]. Similarly, vitamin D deficiency was found to be highly prevalent in North African countries [25] and in healthy Egyptian adolescent girls [26]. Studies in Turkey and Jordan also showed a strong relationship with clothing. Serum 25(OH) D3 levels were highest in women who wore Western clothing, decreasing in traditional women wearing a *hijab* (veil that covers the whole head except the face), and the lowest levels were measured in completely veiled women who wore a *niqab* (additional veil covering the whole face, or all of it except the eyes) [27, 28].

Patients with SLE may be at a higher risk of vitamin D deficiency because of sun avoidance and the frequent use of sunscreens. In addition, many drugs commonly used in disease management, such as glucocorticoids and hydroxychloroquine, might interfere with vitamin D metabolism and determine changes in the serum levels of 25(OH) D3 [29–31]. In adult patients with SLE, the prevalence of vitamin D deficiency/insufficiency varied widely in previous studies, from 8 to 67 % [31–34]. Some studies have documented an association between higher disease activity and a low level of vitamin D [34, 35]. Moreover, a significant negative correlation between 25(OH) D3 and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was noted in European patients [16]. However, no association between disease activity and 25(OH) D3 was observed in other studies [36–38].

Regarding studies on Egyptian SLE patients, one study estimated that insufficient and deficient 25(OH) D3 serum levels among adults with SLE at baseline were 69 and 39 %, respectively [39]. Another study suggested the association of higher SLE disease activity with VDR genes polymorphisms, haplotypes, and decreased 25(OH) D3 levels [40]. There are scanty data on the prevalence of vitamin D deficiency and its relation to disease activity among patients with juvenile-onset systemic lupus erythematosus (JoSLE). The aim of this study was, therefore, to study vitamin D status and its relation to disease activity and parameters in Egyptian patients with JoSLE.

Materials and methods

Subjects

Seventy patients with JoSLE were enrolled in this study. All participants met the revised criteria of the American College of Rheumatology (ACR) for classification of SLE [41] and were being followed up by the Pediatric Rheumatology

Clinic, Cairo University Hospitals, Egypt. In addition, 40 healthy children with matched age, sex, and body mass index (BMI) were recruited as a control group. Serum samples were prospectively collected for the measurement of 25(OH) D3.

Information regarding the medical history, clinical symptoms, and signs was registered at the time of serum sampling. Each patient was also assayed for blood cell count, sedimentation rate, routine chemistry, urinalysis, complement, and anti-dsDNA antibody. Disease activity of SLE was evaluated according to the SLEDAI score [42]. To exclude factors that may influence 25(OH) D3 serum levels, our study was performed during spring, from March to May 2014. Children with JoSLE who had serum aspartate aminotransferase or serum alanine aminotransferase levels that were elevated more than twofold of upper limit of normal, and children with metabolic bone disease or receiving regular anticonvulsant drugs were excluded from the study. Parents of all subjects provided informed consent prior to the participation, and the institutional ethical committee approved the study.

25(OH) D3 assay

The 25(OH) D3 was determined by enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik, USA), according to the manufacturer's instructions. Serum 25(OH) D3 levels ≤ 30 and ≤ 10 ng/mL were defined as vitamin D insufficiency and vitamin D severe deficiency, respectively. The intra-assay coefficient of variation (CV) of 25(OH) D3 was 4.9 %, and the inter-assay CV of 25(OH) D3 was 7.8 % [43].

Statistical analysis

The statistical analysis was conducted using the software SPSS, version 13.0 (Chicago, IL, USA). The data were expressed as mean \pm SD. A *P* value of <0.05 was regarded as significant. Serum 25(OH) D3 results of patients and controls were tested for differences by an independent sample *t* test. Chi-square test was used for comparison of the frequency of vitamin D severe deficiency/insufficiency between patients with SLE and controls. Mann–Whitney test was used for comparison of serum 25(OH) D3 levels according to the clinical manifestations in SLE. Spearman correlation coefficients were calculated to signify the association between different quantitative variables.

Results

Among the 70 JoSLE patients, 49 (70 %) were females and 21 (30 %) were males compared to 25 (62.5 %) and 15 (37.5 %) in the control group. The mean age of patients and controls was 13.1 ± 3.0 and 13.3 ± 3.6 years, respectively.

Disease duration ranged between 0.2 and 12 years with a mean of 3.9 ± 3.0 years. Table 1 summarizes some demographic data, main clinical manifestations, and the current medications of our study population.

Regardless of vitamin D supplementation (97.1 % of patients and none in controls), the mean level of serum 25(OH) D3 was 12 ± 3.7 in JoSLE patients compared to 21 ± 3.5 ng/mL in controls ($P < 0.001$). Twenty-eight of the JoSLE patients (40 %) has 25(OH) D3 severe deficiency (≤ 10 ng/mL) compared to none in the control group ($P < 0.001$); the remaining forty-two of the JoSLE patients (60 %) has 25(OH) D3 insufficiency (≤ 30 ng/mL) compared to 39 (97.5 %) in the control group. None of the JoSLE patients and only one of the controls (2.5 %) has 25(OH) D3 level >30 ng/mL (Fig. 1).

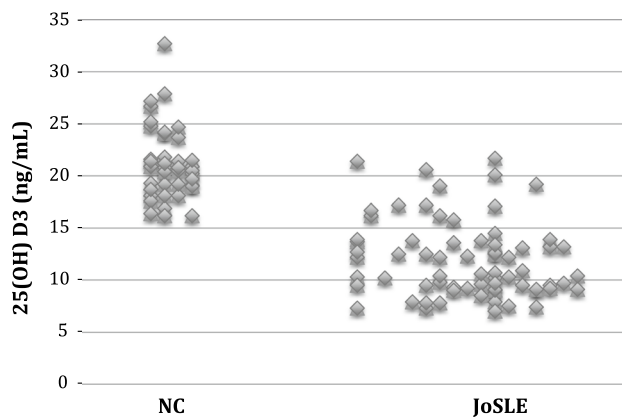


Fig. 1 Serum 25(OH) D3 levels in 40 normal controls (NC) and 70 Juvenile-onset systemic lupus erythematosus (JoSLE)

Table 1 Demographic data of study population, the main clinical manifestations, some laboratory parameters, and current medications of 70 Egyptian patients with JoSLE

Variable	Results	
	Patients	Controls
Weight, mean \pm SD (kg)	44.9 \pm 13.8	46.8 \pm 14.2
Height, mean \pm SD (cm)	150 \pm 20	153 \pm 18
BMI (kg/cm ²)	21.4 \pm 5.9	22.2 \pm 6.1
Routine users of sunscreen	45/70 (64.2 %)	None
Number of patients using hijab/niqab	6/0	4/0
Current glucocorticoid users	68/70 (97.1 %)	
Glucocorticoid dose (mg)	11.8 \pm 5.6	
Current antimalarial users	70/70 (100 %)	
Main manifestation of the disease		
Mucocutaneous	54 (77.1 %)	
Arthralgia and arthritis	23 (32.9 %)	
Nephritis	43 (61.4 %)	
Hematologic	50 (71.4 %)	
Neuropsychiatric	14 (20 %)	
SLE Disease Activity Index (mean \pm SD)	13.2 \pm 9.1	
Laboratory parameters		
ESR (mm/h)	55.4 \pm 34.7	
Serum creatinine (mg/dL)	0.8 \pm 0.7	
C3 (mg/dL)	89.5 \pm 35.5	
C4 (mg/dL)	19.2 \pm 14.1	
Current immunosuppressive users	52/70 (74.3 %)	
Methotrexate	3/70 (4.3 %)	
Azathioprine	18/70 (25.7 %)	
Mycophenolate	21/70 (30 %)	
Cyclophosphamide	10/70 (14.3 %)	
Current vitamin D supplementation (400 IU)	68/70 (97.1 %)	

BMI body mass index, ESR erythrocyte sedimentation rate, C3 complement fragment 3, C4 complement fragment 4

Table 2 Correlations of serum levels of 25(OH) D3 and some demographic data, drug used and some laboratory parameters of the study population

Variable	RHO	P
Age	0.08	0.51
Age at diagnosis	-0.08	0.50
Weight (kg)	-0.10	0.42
Height (cm)	-0.14	0.26
BMI (kg/m ²)	-0.09	0.48
Disease duration (yrs)	0.09	0.47
Cumulative steroid dose	-0.07	0.63
Antimalarial use duration	0.09	0.53
Azathioprine use duration	0.11	0.60
Mycophenolate use	-0.20	0.37
Cumulative cyclophosphamide dose	0.00	0.99
Hb	0.09	0.45
Platelet count	0.06	0.60
Total leukocytic count	0.07	0.59
Lymphocyte count	0.03	0.81
ESR	0.05	0.68
C3 level	0.14	0.26
C4 level	0.06	0.60
Serum creatinine	0.14	0.26
SLE Disease Activity Index (SLEDAI) Score	-0.07	0.55

rho = Spearman correlation coefficient

There was no significant correlation between serum levels of 25(OH) D3 and the demographic data, medication used, and some laboratory data of patients with JoSLE. Disease activity score in our patients was insignificantly correlated with serum levels of 25(OH) D3 (Table 2).

When patients were classified according to the presence or absence of the main clinical manifestations or laboratory parameters of the disease, we found no statistical

Table 3 Statistical significance of serum 25(OH) D3 according to the presence or absence of the main disease manifestations and some laboratory parameters

Variable	Serum 25(OH) D3 levels, mean (SD), ng/mL	<i>P</i>
Main manifestation of the disease		
Mucocutaneous		
Yes	11.7 ± 3.7	0.67
No	12.1 ± 3.7	
Arthralgia and arthritis		
Yes	12.0 ± 3.9	0.99
No	12.0 ± 3.7	
Nephritis		
Yes	11.9 ± 3.8	0.89
No	12.1 ± 3.7	
Hematologic		
Yes	11.7 ± 3.7	0.65
No	12.1 ± 3.8	
Neuropsychiatric		
Yes	12.6 ± 4.4	0.42
No	11.8 ± 3.5	
Anti-dsDNA		
Positive	11.6 ± 3.4	0.09
Negative	13.5 ± 8.1	
Creatinine		
High	13.2 ± 4.5	0.32
Normal	11.8 ± 3.6	
C3		
Low	10.9 ± 3.3	0.06
Normal	12.6 ± 3.9	
C4		
Low	11.1 ± 3.1	0.11
Normal	12.5 ± 4.0	

differences between serum levels of 25(OH) D3 and the presence or absence of the main disease manifestations: mucocutaneous, arthralgia and arthritis, nephritis, hematologic, or neuropsychiatric. In addition, there was no statistically significant difference between serum levels of 25(OH) D3 in JoSLE patients classified according to the anti-dsDNA positivity, high or normal creatinine levels, and low or normal C3 and C4 (Table 3).

Discussion

The aim of this study is to examine vitamin D status in Egyptian JoSLE patients and the potential relationships between vitamin D and disease activity. We evaluated the serum levels of 25(OH) D3 and compared them with clinical features and markers of disease activity.

Vitamin D status has been studied in most countries throughout the Middle East and North Africa [12–28]. These studies revealed that vitamin D deficiency was prevalent across all age groups, geographic regions, and seasons. Thus, it seems that this area of the world is facing today what is, in fact, a new endemic disease. The actual percentage of people with vitamin D deficiency/insufficiency seems to be far greater than reported [44]. In this study, only one out of the 40 control children (2.5 %) has serum 25(OH) D3 level above 30 ng/mL, while 39/40 of the controls (97.5 %) have serum 25(OH) D3 insufficiency (>10–30 ng/mL).

Vitamin D has many regulatory effects on the immune system, and the evidences linking vitamin D status as a potential environmental factor involved in autoimmune disease continue to accumulate. Many studies confirmed the link between vitamin D status and insulin-dependent diabetes mellitus, multiple sclerosis, inflammatory bowel diseases, and rheumatoid arthritis [45, 46]. Many factors may contribute to higher risk of vitamin D deficiency/insufficiency in patients with SLE. These include the sun avoidance, the frequent use of sunscreens, and the common use of drugs that may interfere with vitamin D metabolism such as glucocorticoids, hydroxychloroquine, and immunosuppressives. In spite of the unexpected results of the levels of 25(OH) D3 in the control group, vitamin D supplementation in 97.1 % of JoSLE patients and the use of the more strict definition of 25(OH) D3 deficiency (≤ 10 ng/mL), i.e., critically low deficiency [34], we found a significant difference between the levels of 25(OH) D3 in JoSLE patients compared to the control group. The mean level of serum 25(OH) D3 was 12 ± 3.7 in JoSLE patients compared to 21 ± 3.5 ng/mL in controls ($P < 0.001$). There was no significant correlation between the different medications used in this study and the level of 25(OH) D3. The effect of glucocorticoids and hydroxychloroquine on the serum levels of 25(OH) D3 cannot be evaluated in this study because 68/70 (97.5 %) of our patients were on glucocorticoids and all patients were on hydroxychloroquine. All the JoSLE patients in this study have either 25(OH) D3 severe deficiency ≤ 10 ng/mL (40 %) or 25(OH) D3 insufficiency ≤ 30 ng/mL (60 %). In contrast, Casella et al. [47] found no significant difference between the serum levels of 25(OH) D3 in 57 patients with JoSLE in Sao Paulo, Brazil, compared to 37 healthy controls. One study estimated that insufficient and deficient 25(OH) D3 serum levels among Egyptian adults with SLE at baseline were 69 and 39 %, respectively [39]. Thus, the problem of vitamin D deficiency/insufficiency in Egyptian patients with SLE seems to be common in both JoSLE and adult patients.

There were controversial results in previous studies regarding the relation between SLE disease activity and the level of 25(OH) D3. Some studies have documented

higher disease activity associated with low level of 25(OH) D3 [34, 35, 47], and other studies found no association between the disease activity and 25(OH) D3 levels [36–38]. In this study, no significant correlation was found between SLE Disease Activity Index (SLEDAI) and the levels of 25(OH) D3. In addition, there was no significant association between the main disease manifestations or laboratory parameters that may reflect the activity or severity of the disease such as sedimentation rate, dsDNA positivity, low complement levels, the presence of nephritis or neuropsychiatric manifestations, and the level of 25(OH) D3.

Limitations of our study include the relatively small size of the control group. Additionally, our center at Cairo University Hospitals mainly serves low socioeconomic class, and most of our controls were recruited from relatives of patients presented to these hospitals. This low socioeconomic status may have an impact on vitamin D status of our patients as well as the so-called healthy controls.

Overall, in spite of the limitations of this study, the results indicate that the serum 25(OH) D3 levels are significantly lower in JoSLE patients compared to the control group, and the problem of vitamin D deficiency/insufficiency in Egyptian patients with JoSLE may be greater than in SLE adult patients. Increased awareness with the issue of vitamin D deficiency/insufficiency in JoSLE among pediatricians and rheumatologists in our area is mandatory. In addition, the use of higher dosage of vitamin D supplementation in patients with JoSLE may be required to ensure adequate level.

Conflict of interest All authors have no conflict of interest in relation to the research topic, and the authors did not receive any funding concerning this research.

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