Serum vitamin D and peripheral T-regulatory cells in systemic lupus erythematosus and their relation with disease activity

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Background
Systemic lupus erythematosus (SLE) patients have a decreased number of T-regulatory cells (Tregs) in peripheral blood. Vitamin D deficiency is prevalent in SLE. Immunomodulatory effects of vitamin D include the expansion of Tregs.

Objectives
The aim of this study was to assess the percentage of Tregs and vitamin D level in SLE and their relation with disease activity.

Patients and Methods
A total of 40 SLE patients underwent evaluation for disease activity using the SLE disease activity index and were tested for the percentage of peripheral Tregs using anti-CD4, anti-CD25, and anti-FOXP3 monoclonal antibodies. Vitamin D was assessed using a commercially available 25-OH VitD-EIA kit. The study also included 40 healthy individuals who served as controls.

Results
SLE patients had lower levels of vitamin D (22.3 ± 7.53) and Treg% (1.95 ± 0.18) in comparison with controls. Patients with active disease had significantly lower levels of vitamin D. However, there was no significant difference between patients with and those without disease activity as regards Tregs. Correlation between vitamin D and various disease parameters showed negative correlation between vitamin D and each of disease activity, creatinine, and urinary protein ($P < 0.05$) and a positive correlation with C_4 ($P < 0.05$). Correlation between Tregs% and various disease parameters showed a significant negative correlation as regards anti-dsDNA ($P < 0.05$). No correlation was detected between Tregs% and vitamin D.

Conclusion
There are decreased levels of vitamin D and Treg% in SLE. Lower levels of vitamin D correlate with disease activity; yet, no correlation between serum vitamin D and Treg% was detected.

Keywords:
systemic lupus erythematosus, T-regulatory cells, vitamin D

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Introduction

Systemic lupus erythematosus (SLE) is a complex multisystem autoimmune disease. Vitamin D deficiency has been proposed as an environmental trigger of disease onset and as a contributor to increased activity in SLE. SLE patients are prone to develop vitamin D deficiency because of photosensitivity, leading to sun avoidance and other sun protective measures. The impact of vitamin D on immune function previously seen *in vitro* and in cross-sectional studies has now been shown in prospective human studies, strengthening the evidence that there is a connection between SLE and vitamin D status [1].

Vitamin D in general exerts an inhibitory action on the adaptive immune response through several mechanisms, including the inhibition of dendritic cell maturation, Th1 activity, B cel1 maturation and differentiation, and function of T-regulatory cells [2].

SLE is characterized by the loss of tolerance to self-antigens and the production of autoantibodies. Many recent studies have suggested that CD4+CD25+ regulatory T cells (Tregs) exhibit regulatory/immunosuppressive activity by actively preventing the activation and effector function of autoreactive T cells that have escaped tolerance, and thus play a critical role in the maintenance of self-tolerance [3].

One possible explanation of the emergence of autoimmunity in SLE could relate to the deficient function of Tregs. Although there are controversial data on the number and function of Tregs in SLE, recent reports have indicated that patients with SLE have a low number of Tregs in their peripheral blood, and many works suggested a defective function of Tregs in this condition [4].

Furthermore, the frequency and function of Tregs might be related to disease activity in SLE. However, the exact defects of Tregs are unknown and the pathogenic role of Tregs in SLE remains to be fully delineated [5].

As patients with SLE, especially those with increased disease activity, were suggested to have decreased vitamin D levels, and because encouraging results have been recently reported suggesting a beneficial role of vitamin D supplementation in patients with SLE, studying serum vitamin D levels in relation to numbers and functions of Treg cells in SLE patients might focus future studies on the potential role of vitamin D in the treatment of SLE [6].

The aim of the work

The aim of this study was to assess the percentage of peripheral Tregs and serum vitamin D levels in patients with SLE and ascertain whether both parameters show any relation with disease activity.

Patients and methods

This study was carried out at the Ain Shams University Hospital in the Departments of Clinical Pathology and Rheumatology. The study included two groups: group I and group II. Group I comprised 40 patients with SLE, diagnosed according to the Revised American College of Rheumatology Criteria for SLE diagnosis [7], and group II comprised 40 age and sex-matched healthy adults as controls.

All patients were subjected to the following:

(1) Careful history taking and thorough clinical and musculoskeletal examination with assessment of disease activity using the SLE disease activity index [8].
(2) Laboratory investigations including:
    (a) complete blood count on a Coulter Counter (Synchron CX9ALX, MININEPH, EPICS XL Beckman flow cytometer);
    (b) renal function tests (blood urea nitrogen (BUN)), serum creatinine, and creatinine clearance, and 24 h urine proteins) performed on a Synchron CX9ALX;
    (c) Antinuclear antibody (ANA) and anti-ds-DNA by indirect immunofluorescence; and
    (d) serum C3 and C4 levels by nephelometry (MININEPH).

All patients and controls were tested for the following:

(1) peripheral Treg cells percentage using anti-CD4 [eBioscience, USA clone (RPA-T4)], anti-CD25 [eBioscience, USA clone (BC96)], and anti-FOXP3 monoclonal antibodies [eBioscience, USA clone (PCH101)], which was performed using an EPICS XL Beckman flow cytometer.
(2) serum levels of 25-OH vitamin D using a commercially available 25-OH VitD-EIA kit (Immundiagnostik; Bensheim and Biomedica, Vienna, Austria).

Statistical methods

Data were statistically described in terms of mean ± SD, range or frequencies (number of cases), and percentages. The χ^2-test was used to compare qualitative variables. The unpaired *t*-test was used to compare two independent groups as regards quantitative variables. The Spearman and Pearson correlation coefficient rank tests were used to rank different variables against each other positively or inversely. Level of significance was determined as follows: *P* greater than 0.05 = not significant (NS); *P* less than 0.05 = significant (S); and *P* less than 0.01 = highly significant (HS).

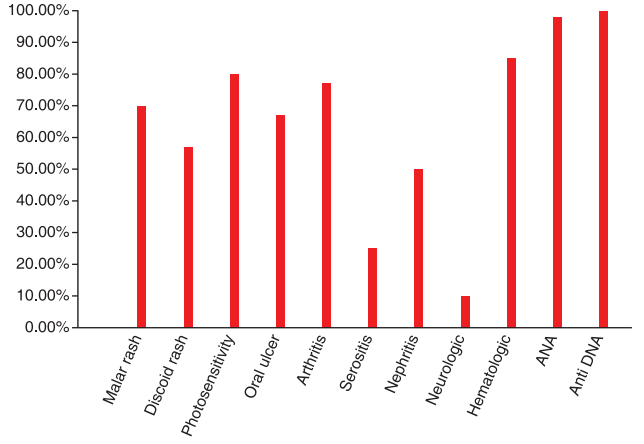
Results

This study included two groups: group I and group II. Group I comprised 40 SLE patients (36 female and four male patients); their ages ranged from 15 to 45 years, with disease duration of 2–13 years. Group II comprised 40 age and sex-matched healthy adults (27 female and 13 male patients), with ages ranging from 17 to 46 years, and were studied as the control group (Fig. 1 and Tables 1 and 2).

Patients in group I were further subdivided on the basis of disease activity into group Ia and group Ib. Group Ia comprised 22 patients with disease activity and group Ib comprised 18 patients without disease activity.

Correlation between serum vitamin D and various disease parameters among SLE patients showed a significant negative correlation between serum vitamin D level and each of disease activity ($r = 0.42$), creatinine

Figure 1



Characteristics of SLE patients. SLE, systemic lupus erythematosus.

(r = 0.34), and urinary protein (r = 0.39). A significant positive correlation was also detected between serum vitamin D and C4 (r = 0.41) (Table 3).

Correlation between Treg% and various disease parameters showed a significant negative correlation between Treg% and anti-dsDNA (r = 0.39). However, no significant correlation was detected between Treg% and serum vitamin D (Table 4).

Discussion

The interaction of vitamin D with the immune system has been the target of a growing number of publications in recent years. Current studies have linked the deficiency of vitamin D with different autoimmune diseases, including SLE [9].

SLE is a T-cell and B-cell-dependent autoimmune disease characterized by the appearance of autoantibodies, a global regulatory T-cell (Tregs) depletion, and an increase in Th17 cells. Recent studies have shown the multifaceted immunomodulatory effects of vitamin D, notably the expansion of Tregs and the decrease in Th1 and Th17 cells [6].

There is a connection between lupus and the disturbance of regulatory T cells that plays an important role in maintaining a healthy immune system. Several studies have demonstrated that decreased numbers and/or function of Tregs contribute to the pathogenesis of SLE [10].

The aim of this study was to assess the frequency of peripheral T-regulatory cells and serum vitamin D levels in patients with SLE and ascertain whether both parameters show any relation with disease activity.

This study was conducted on 40 SLE patients (36 female and four male patients); their ages ranged from 15 to 45 years, with disease duration of 84 ± 36 months. Of them, 22 patients had active disease and 18 patients were inactive. Clinical manifestations included arthritis (77%), photosensitivity (80%), malar rash (70%), and nephritis (50%). All patients were anti-DNA positive and 85% showed hematological abnormalities.

This study showed that SLE patients had significantly lower levels of vitamin D (22.3 ± 7.53) compared with the control group (35.8 ± 8.61). Treg% was also significantly decreased in SLE patients (1.95 ± 0.18) compared with controls (3.82 ± 0.28). Patients with active disease had significantly lower levels of serum vitamin D (19.4 ± 5.23) than did patients with inactive disease (26.84 ± 3.59). However, there was no significant difference between patients with and those without disease activity as regards Treg% (1.65 ± 0.47 vs. 1.82 ± 0.95, respectively).

In this study, correlation between serum vitamin D and various disease parameters showed a significant negative correlation between serum vitamin D level and each of disease activity, creatinine, and urinary protein (P < 0.05). A significant positive correlation was also detected between serum vitamin D and C_4 (P < 0.05). Correlation between Treg% and various disease parameters showed a significant negative correlation between Treg% and anti-dsDNA (P < 0.05). However,

Table 1 Comparison between group I and II as regards serum vitamin D and treg%

Variable	Mean ± SD		*P* value	Significance
	Group I	Group II		
Serum vitamin D (ng/ml)	22.3 ± 7.53	35.8 ± 8.61	<0.01	HS
Treg%	1.95 ± 0.18	3.82 ± 0.28	<0.05	S

HS, highly significant; S, significant.

Table 2 Comparison between group Ia and Ib as regards serum vitamin D and treg%

Variable	Mean ± SD		*P* value	Significance
	Group Ia	Group Ib		
Serum vitamin D (ng/ml)	19.4 ± 5.23	26.84 ± 3.59	<0.05	S
Treg%	1.65 ± 0.47	1.82 ± 0.95	>0.05	NS

NS, nonsignificant; S, significant.

Table 3 Correlation between serum vitamin D and various disease parameters

Parameters	Serum vitamin D (ng/ml)		
	r	*P*	Significance
Disease activity	−0.42	<0.05	S
Treg%	0.12	>0.05	NS
Creatinine	−0.34	<0.05	S
Urinary protein	−0.39	<0.05	S
C3	0.11	>0.05	NS
C4	0.41	<0.05	S

NS, nonsignificant; S, significant.

Table 4 Correlation between Treg% and various disease parameters

Parameters	Treg%		
	r	*P*	Significance
Disease activity	−0.17	>0.05	NS
Serum vitamin D	0.12	>0.05	NS
Anti-dsDNA	−0.39	<0.05	S
C3	0.16	>0.05	NS
C4	0.20	>0.05	NS

NS, nonsignificant; S, significant.

no significant correlation was detected between Treg% and serum vitamin D.

Vitamin D deficiency is more prevalent among SLE patients than in the general population. Over the past decade, many studies across the globe have been carried out to investigate the role of vitamin D in SLE from various clinical angles, and several studies have reported an association between vitamin D deficiency and the risk of SLE [11,12].

Furthermore, other studies recently reported a positive association between SLE activity and vitamin D deficiency [13,14]. In a study including 95 patients with SLE, the levels of vitamin D were significantly lower in patients with active SLE (n = 41; 43%) than in those with inactive disease (n = 54; 57%; P = 0.04). The mean levels were 22.3 (14) nmol/l for patients with active disease against 25.0 (14) nmol/l for patients with inactive SLE. Similar to the present study, a significant negative correlation existed between vitamin D and anti-dsDNA (r = −0.38; P < 0.001) and a positive correlation with C4 (r = 0.25; P = 0.25) [15].

In contrast, other studies found no relation between vitamin D and disease duration, the SLE disease activity index, or SLICC-ACR index in SLE patients [16]. In a recent study, although a high prevalence of vitamin D deficiency was found in patients with SLE (57.7%), no association between vitamin D and the clinical variables and laboratory tests was detected [17].

Because lymphocytopenia is a characteristic hematological feature of SLE, the percentage of Tregs is more important than the absolute number of Tregs [18]. In this study the percentage of Tregs was significantly decreased in patients with SLE.

Previous studies have found a significant decrease in circulating Treg cells in the peripheral blood of SLE patients compared with controls. This finding was attributed to the role of Treg cells in autoimmune diseases, especially in SLE, which showed that the pathogenesis of SLE involves the breakdown of immunologic self-tolerance resulting in the development of autoantibodies. Many T-cell and B-cell abnormalities have been described, and these include defects in regulatory T cells that normally prevent pathologic self-reactivity [19,20].

Supportive evidence to these explanations stated that in human SLE the Treg cells can directly suppress autoantibody-producing B cells, including those that belong to pathogenic subtypes that are found expanded in active SLE. The cell-to-cell-dependent mechanisms of suppression of autoreactive B cells by Treg cells in SLE, which could represent an attempt to directly control humoral autoimmunity, involve the release of perforin and granzyme by activated Treg cells and the induction of apoptosis in these autoreactive B cells [21].

In addition, studies have shown a decrease in circulating Treg number in SLE patients, especially during the flare [2]. Furthermore, a study stated that there was a decreased percentage of Tregs in SLE patients (15.5 ± 0.2%, P = 0.002) compared with healthy controls (22.1 ± 0.9%) and that the number of Tregsbright was negatively correlated with the levels of anti-dsDNA antibody and the number of CCR4+ Tregsbright had a positive correlation with the levels of C3 [5].

In contrast, another study reported no significant difference as regards the number of $CD4^+CD25^+$ T cells in SLE patients compared with healthy controls and also detected no significant correlation between disease activity and the number of Tregs [22], which was consistent with the results of the present study in which, although reduced numbers of Tregs were detected in SLE patients, no significant correlation with disease activity was detected. These discrepancies may arise from fair differences between isolation protocols and flow cytometry technicalities and differences in the levels of disease activity of the studied patients.

Although the immunoregulatory role of vitamin D has been the object of a growing number of studies in patients with SLE, in the present study no significant correlation was detected between serum vitamin D and Treg% in contrast to other studies in which a significant positive correlation between vitamin D and Treg migratory capacity was detected [2].

Finally, the present study detected significantly lower levels of serum vitamin D and Treg% in SLE patients. Patients with disease activity had significantly lower levels of serum vitamin D; yet, levels of Treg% showed no significant difference between active and inactive groups. There was no correlation between Treg% and disease activity, and no correlation between serum vitamin D and Treg% was detected. In conclusion, it remains unclear whether vitamin D levels in SLE patients crucially influence Treg%, and the role of Tregs in the pathogenesis of SLE still remains to be further investigated.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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