Study the relationship between vitamin A deficiency, T helper 17, regulatory T cells, and disease activity in patients with systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease in which activation of the innate immune system with activation of T and B lymphocytes and autoantibodies production, immune complex deposition, and complement-induced tissue damage occurs. It is characterized with relapsing-remitting course, which occurs at any age, but it has poorer outcome in childhood than adult patients. Its manifestations vary from mild muco-cutaneous to severe life-threatening illness [1].

Pathogenesis of SLE is still unclear, but balance between T helper 17 (Th17) and regulatory T cells (Tregs) underlies the outcome of the disease [2]. The Tregs, a subset of CD4+T cells, are the main regulators of immune response and suppress inflammatory T-cell responses. Th17, a newly discovered subset of CD4+ T cells, is identified according to its ability to produce interleukin (IL)-17A, IL-17F, and IL-22. It has been shown that there is a positive correlation between the number of Th17 and organ damage, whereas there is a negative correlation between Treg and flares in patients with SLE. Thus, the balance between the two cells can be a new therapeutic option in patients with SLE [3].

The cytokines produced by the activated immune system in patients with SLE also suppress the generation of natural Treg cells and promote a proinflammatory T-cell response dominated by Th17 cells [4].

Background

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with activation of the innate and adaptive immune systems. Vitamin A deficiency causes imbalance of T helper 17 (Th17) and regulatory T cell (Treg), deteriorating the progression of SLE.

Aim

To determine the relationship between vitamin A levels and Th17 and Treg level in patients with SLE and its relation to disease activity.

Patients and methods

A total of 45 female patients with SLE diagnosed according to the American College of Rheumatology criteria and 45 healthy age-matched and sex-matched patients as control group were included. Full assessment was done including medications, clinical examination (pain evaluation by visual analogue scale and assessment of disease activity by SLE disease activity index), laboratory investigations, and albumin–creatinine ratio. Serum levels of vitamin A were measured by a human KAMIYA kits, and flow cytometry was used for measuring Th17 and Treg percentages.

Results

There was a significant deficiency of vitamin A level in patients with SLE compared with controls ($P=0.001$). There was a significant negative correlation between vitamin A and Th17 ($P=0.001$) and positive correlation between vitamin A and Treg percentages ($P=0.001$). There was a negative correlation between vitamin A levels and albumin–creatinine ratio in patients with SLE ($R=-0.255$). A positive correlation between serum levels of vitamin A and C3 and C4 was found ($P=0.001$).

Conclusion

Vitamin A deficiency is a bad prognostic factor in patients with SLE, affecting Th17/Treg balance. Routine use of retinoic acid may be a promising supplementary agent in patients with SLE, improving its prognosis.

Keywords:

systemic lupus erythematosus, Th17/Treg balance, vitamin A

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The active metabolite of vitamin A (retinoic acid) has various biologic properties, including its important role in modulating Th17 and Treg balance, as it has the ability to decrease proliferation and IL-6 induction of Th17 while promote the proliferation of Treg; moreover, it enhances transforming growth factor-β signaling in Treg by increasing the phosphorylation of Smad3 [5].

So the use of vitamin A and retinoic acid may be a promising supplementary agent regulating the balance of Th17 and Treg [5].

The aim of this study was to determine the correlation between vitamin A levels and Th17 and Treg populations in patients with SLE and its relation to disease activity.

**Patients and methods**

This hospital-based case–control study was conducted on 45 female patients with SLE recruited from the outpatient clinic of Rheumatology, Physical Medicine and Rehabilitation department, Menoufia University, between August 2017 and October 2018. All patients fulfilled at least four criteria of the updated American College of Rheumatology criteria [6] with age between 23 and 55 years, presented with active disease, characterized by systemic lupus erythematosus disease activity index (SLEDAI) score more than 3 [7] and did not take vitamin A supplementation. Moreover, 45 healthy age-matched and sex-matched controls and who did not take vitamin A supplementation were recruited. Patients with severe infection or immunodeficiency, patients with features of vitamin A deficiency, and patients with thyroid abnormalities, chronic liver, or renal disease were excluded from this study.

The ethical committee guidelines in Menoufia University were followed, and informed consent was taken from each patient of the study. Full history taking; general examination; local joint examination, with pain evaluation using visual analogue scale [8]; and laboratory investigations, including complete blood count, erythrocyte sedimentation rate, renal function tests (serum creatinine and blood urea), liver enzymes (alanine aminotransferase and aspartate aminotransferase), C-reactive protein (high sensitive), albumin–creatinine ratio, antinuclear antibody, anti-double stranded DNA titer (anti-dsDNA), and C3 and C4 were done. SLEDAI was used to assess the disease activity [7]. Measurement of the serum vitamin A level was done using the vitamin A human KAMIYA biomedical company kits, and flow cytometry also was done for measuring Th17 and Treg percentages [9].

Overall, 15 ml of blood sample was collected from each individual under aseptic conditions. It was divided into three parts: (a) 8 ml was added to EDTA-contained sterile tubes for the determination of complete blood count and CD markers;(b) 5.4 ml was added to sterile plain tubes for assessment of vitamin A level, C-reactive protein, and serum creatinine; and (c) 1.6 ml of blood was delivered into a tube containing 0.4 ml trisodium citrate for erythrocyte sedimentation rate.

The concentrations of serum vitamin A were determined by enzyme-linked immunosorbent assay. A quantitative sandwich enzyme immunoassay technique that measures human vitamin A was used. Standards and samples were sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for vitamin A, which was recognized by a streptavidin–peroxidase conjugate. All unbound material was then washed away, and a peroxidase enzyme substrate was added. The color development was stopped, and the intensity of the color was measured [10].

Peripheral blood mononuclear cells were isolated from 8 ml of whole blood samples. Blood was mixed with an equal volume of PBS (pH: 7.4), layered on Ficoll reagent (1077 g/ml), and centrifuged at 1800g for 20 min. The buffy coat containing mononuclear cells was recovered, mixed with 20 ml PBS, and centrifuged at 3000g for 10 min, and then the supernatant was discarded. Washed cells were incubated with labeled monoclonal antibody which binds to cells expressing the antibody of interest and then the cells expressing monoclonal were fluorescently stained.

For each sample, three tubes were prepared, one for the unstained sample (auto control), and 100 ml of peripheral blood mononuclear cells at 5×10^5/tube WAS stained in two tubes: one for Treg with PE-anti-CD4 plus anti-CD25 FITC (fluorescein isothiocyanate) and the other with PE-anti-CD4 for 30 min. Then in both tubes, cells were fixed followed by intracellular staining with PerCP-anti-FoxP3 in first tube and anti-IL17 FITC in the second tube. After incubation for 30 min at 4°C and subsequently washing twice with PBS, the cells were suspended in 300μl of PBS for final flow
cytometry analysis. The percentages of Treg (CD4 +CD25+FoxP3+) and T helper 17 (CD4+ anti-IL17 +) cells in all samples were determined by flow cytometry, using FACS Calibur flow cytometer (BD Immune Cytometry System, San Jose, California, USA).

Statistical analysis
Data were described as mean±SD. SPSS version 17.0 (IBM Corporation, Chicago, USA) for Windows was used for statistical analysis and generation of graphs. z-Test and Pearson correlation were used [11].

Results
A total of 45 female patients with SLE with a mean age of 36.6 years (range: 23–55) and 45 healthy age-matched and sex-matched controls with a mean age of 34.36 years were included in this study (Fig. 1).

Hypovitaminosis A was considered if up to 30 mcg/dl. It was observed in 35 patients with SLE (77.77%) and was normal in all the control group. Vitamin A levels in the patients with SLE were significantly lower compared with controls (P=0.001; Table 1).
There was a significant increase in SLEDAI in patients with SLE with low vitamin A levels compared with those with normal vitamin A levels ($P=0.001$; Table 2 and Fig. 2).

The was a statistically significant negative correlation between vitamin A level and Th17 level ($P=0.001$, $R=-0.852$) and a significant positive correlation between vitamin A and Treg populations ($P=0.001$, $R=0.882$; Table 3 and Graphs 1 and 2.

A statistically significant positive correlation between vitamin A and C3 and C4 ($P=0.001$, $R=0.642$, and $R=0.778$) was found, with a negative correlation between vitamin A levels and albumin–creatinine ratio in patients with SLE. Moreover, there was a statistically significant negative correlation between

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**Graph 1**

![Graph showing a significant positive correlation between serum level of vitamin A and Treg populations. Treg, regulatory T cell.](image1)

**Graph 2**

![Graph showing insignificant negative correlation between vitamin A and Th17 populations. Th17, T helper 17.](image2)

**Graph 3**

![Graph showing negative correlation between vitamin A level and albumin–creatinine level.](image3)

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**Table 1** Vitamin A level and age of patients with systemic lupus erythematosus and control group

|                      | Patients with SLE ($N=45$) | Control group ($N=45$) | $t$-Test | $P$ value |
|----------------------|-----------------------------|------------------------|----------|-----------|
| Age (mean±SD)        | 36.62±9.19                  | 34.36±9.57             | 1.15     | 0.26      |
| Vitamin A, Mean±SD   | 21.52±10.58                 | 51.22±10.9             | 13.1     | 0.001     |
| Range                | 8.7–41                      | 33.3–62.5              |          |           |

SLE, systemic lupus erythematosus.

**Table 2** Systemic lupus erythematosus disease activity index in patients with systemic lupus erythematosus with low vitamin A level and those with normal vitamin A level

| SLEDAI                      | Group                        | Likelihood ratio | $P$ value |
|-----------------------------|------------------------------|------------------|-----------|
|                             | Low vitamin A level patients | Normal vitamin A level patients |             |
| $n$ (%)                     | 35 (77.77)                   | 10 (22.2)        | 72.6      | 0.001HS   |
| Means±SD                    | 10.36±2.95                   | 3.10±1.20        | $t=14.46$ | 0.001HS   |

SLEDAI, systemic lupus erythematosus disease activity index.
Table 3 Correlation of vitamin A levels with T helper 17 and regulatory T cell percentages and C3, C4, anti-double stranded DNA titer and albumin–creatinine ratio in patients with systemic lupus erythematosus

| Vitamin A                        | r      | P value |
|----------------------------------|--------|---------|
| Th17 level                       | -0.852 | 0.001HS |
| Treg level                       | 0.882  | 0.001HS |
| Albumin–creatinine ratio (mg/g)  | -0.255 | 0.091   |
| C3                               | 0.642  | 0.001HS |
| C4                               | 0.778  | 0.001HS |
| Anti-dsDNA titer                 | -0.708 | 0.001HS |

Anti-dsDNA, anti-double stranded DNA; Th17, T helper 17; Treg, regulatory T cell.

Table 4 Comparison between patients with SLE with hypovitaminosis A with those with normal vitamin A level regarding C3, C4, regulatory T cell 17, T helper 17, anti-double stranded DNA titer, and albumin–creatinine ratio

| Vitamin A                        | Patients with low vitamin A (N=35) | Patients with normal vitamin A (N=10) | t-Test | P value |
|----------------------------------|------------------------------------|--------------------------------------|--------|---------|
| C4                               | 11.36±4.15                         | 16.16±5.18                           | 5.47   | 0.001   |
| C3                               | 99.98±46.4                         | 19.5±33                              | 11.89  | 0.001   |
| Albumin–creatinine ratio (mg/g)  | 73.00±54.71                        | 16.18±6.32                           | 6.92   | 0.001   |

Anti-dsDNA, anti-double stranded DNA; Th17, T helper 17; Treg, regulatory T cell.

vitamin A and anti-dsDNA titers (P=0.001, R=-0.708; Table 3 and Figure 3 and Graph 3).

There were statistically significant differences between patients with SLE with hypovitaminosis A compared with those with normal vitamin A level regarding C3, C4, Treg17, Th17, anti-dsDNA titer, and albumin percent (P=0.001; Table 4).

Discussion

SLE is a multisystem autoimmune disease that causes persistent inflammation and multiple organ damage. SLE is initiated by imbalance of Th17 and Treg populations [2]. Recent studies showed that increase number of Th17 and reduction of Treg were strongly associated with organ damage and clinical symptoms of SLE [12]. Vitamin A has a major role in immune system regulation especially Th17 and Treg balance [5].

This study showed a significant increase for the incidence of hypovitaminosis A in patients with SLE compared with healthy controls (P=0.001). This comes in agreement with Kida et al. [13] who reported decreased vitamin A levels in patients with SLE. This significant low level of vitamin A is explained by the chronic inflammatory conditions, which decrease the expression of the lecithin retinol acyltransferase enzyme causing disruption of vitamin A storage in the liver.

D’Ambrosio et al. [14] also reported low levels of vitamin A in patients with SLE compared with healthy controls. They explained that by the interference of lecithin retinol acetyl-transferees gene, which may be associated with vitamin A deficiency.

This study showed that the patients with low vitamin A levels have higher disease activity (SLEDAI). This comes in agreement with Hanly et al. [15] as they reported high disease activity in vitamin A-deficient patients as compared with those with normal vitamin A levels due to immuno-regulatory imbalance which caused by vitamin A deficiency.

This study reported the association between vitamin A deficiency and high titer of Th17, albumin creatinine ratio, and anti-dsDNA titer (P=0.001). Moreover, vitamin A deficiency is associated with low titer of Treg, C3, and C4 (P=0.001).

In accordance to our study, Elias and his colleagues reported the significant positive correlation between vitamin A and Treg and negative correlation between vitamin A and Th17. This is owing to the ability of ATRA and other agonists of the retinoic acid receptor alpha (RARα) to inhibit the formation of Th17 cells and promote Treg differentiation [16].

Our results also come in agreement with Nolting et al. [17] and Sobel et al. [18] who reported the significant positive correlation between Treg and vitamin A levels and the negative correlation between it and Th17. They reported that vitamin A can enhance the conversion of naive T cells into Treg and also inhibit Th17 differentiation by inhibiting IL-6 and IL-23 receptor expression.

The negative correlation between serum vitamin A levels and albumin–creatinine ratio in patients with SLE (P=0.091, R=-0.255) was supported by Bennet et al. [19] who reported the high levels of albumin–creatinine ratio when vitamin A decreases.

A significant negative correlation between vitamin A levels and anti-dsDNA titer was reported in our study, which is reported also by Hanly et al. [15].
The significant positive correlation between vitamin A deficiency and low levels of C3 and C4 in patients with high disease activity in our study was supported by Hanly et al. [15], who reported the association between vitamin A deficiency and complement consumption and low complement levels in SLE patients with high disease activity.

Our study reported a significant increase in Th17 and decrease in Treg in patients with SLE with low vitamin A levels compared with those with normal vitamin A levels (P=0.001). This comes in agreement with Pan et al. [12].

Szmyrka-Kaczmarek et al. [2] reported high Th17 and low Treg levels in vitamin A-deficient SLE patients compared to those with normal vitamin A levels. Low level of Treg caused increased autoantibodies productions, tissue damages, and clinical deteriorations in patients with SLE.

In contrast, Yang et al. [20] reported the elevation of both Th17 and Treg populations in patients with SLE. The increased Treg may be a compensatory mechanism to autoimmune processes, such as increase Th17 subset. In this study, we excluded patients with immunodeficiency or those associated with other autoimmune disorders, and our patient groups were larger.

This study also reported significant decreased of C3 and C4 levels in vitamin A-deficient patients compared with those with normal levels (P=0.001). This is owing to high disease activity in vitamin A-deficient patients and more complement activation causing decreased C3 and C4. Szmyrka-Kaczmarek et al. [2] reported this decrease in C3 and C4 in patients with active SLE.

In vitamin A-deficient patients, the albumin-creatinine ratio (>30 mg albumin/creatinine) and anti-dsDNA titer were higher than patients with normal levels. This proves the severity of organ damage especially the kidneys in vitamin A-deficient patients with SLE owing to immune disruption, autoantibodies production, and immune complex deposition. Bennet et al. [19] reported the same results.

**Conclusion**

Vitamin A deficiency is a bad prognostic factor in patients with SLE, affecting Th17/Treg balance, as it can inhibit Th17 differentiation and enhance Treg differentiation. Treatment with vitamin A is a promising supplementary option and may provide improvement in SLE prognosis.

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**Conflicts of interest**

There are no conflicts of interest.

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