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The changes of Treg and Th17 cells relate to serum 25(OH)D in patients with initial-onset childhood systemic lupus erythematosus

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Background: T helper 17 (Th17) cells and regulatory T cells (Treg) are known to play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). Improving the balance between Treg and Th17 cells can be a promising new therapeutic target in SLE patients. Vitamin D has a significant impact on the immune inflammatory process and the immune cells involved in this process. The purpose of this study is to investigate the relationship between Th17, Treg, cytokines, and serum 25 hydroxyvitamin D [25(OH)D] in patients with initial-onset childhood SLE.

Methods: A total of 82 children aged <18 years with initial-onset SLE were included, as well as 60 healthy subjects during the same period at the Pediatrics Department of the Second Hospital of Hebei Medical University. The chemiluminescence method was performed to detect serum 25(OH)D levels. Flow cytometry was used to evaluate Treg and Th17 cells. An enzyme-linked immunosorbent assay kit was used to evaluate plasma interleukin (IL)-23, IL-17, IL-10, IL-6, and tumor necrosis factor alpha (TNF-α) concentrations.

Result: The serum 25(OH)D levels in patients with initial-onset childhood SLE were significantly lower than those in the healthy controls. The proportion of lupus nephritis (LN) was higher in the vitamin D insufficiency group (71.4%) compared with the vitamin D sufficiency group (30.3%) ($p < 0.05$). The SLE disease activity index (SLEDAI) was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$).The 25(OH)D level was positively correlated with the Treg ratio ($r = 0.337$, $p = 0.002$), and it was negatively correlated with the Th17 cell ratio ($r = -0.370$, $p = 0.001$). The serum 25(OH)D level had a negative correlation with IL-23 ($r = -0.589$, $p < 0.001$), IL-17($r = -0.351$, $p = 0.001$), TNF-α ($r = -0.283$, $p = 0.01$), IL-6 ($r = -0.392$, $p < 0.001$), and IL-10 ($r = -0.313$, $p = 0.004$) levels.

Conclusion: The serum 25(OH)D levels decreased in patients with initial-onset childhood SLE. There was a negative correlation between the serum 25(OH)D levels and SLEDAI. The serum 25(OH)D levels in patients with initial-onset childhood SLE were negatively correlated with the Th17 ratio and related cytokines, while positively correlated with the Treg ratio.

KEYWORDS

25(OH)D, SLE, Treg, Th17, cytokines

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that causes chronic inflammation and damages multiple tissues and organs, including the central nervous system, skin mucosa, cardiovascular system, kidneys, and joints. The pathogenesis of SLE is not fully understood. SLE is characterized by polyclonal activation of T and B lymphocytes. In addition to an imbalance of T helper 1 (Th1) and T helper 2 (Th2) cells, the regulatory T cells (Treg) and T helper 17 (Th17) cells are known to play a crucial role in the pathogenesis of SLE (1). Studies have found that quantity anomalies or/and functional defects of Treg and Th17 cells were associated with flares and organ damages in SLE patients (2). Th17 cells secrete a profile of potent pro-inflammatory cytokines, including interleukin-17 (IL-17), and potent tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) upon certain stimulation (3). Interleukin-23 (IL-23) is an important cytokine that promotes the secretion of interleukin-17 by Th17 cells to maintain pathological status by combining with IL-23 receptor (4). Although it is widely believed that Treg cells play a preventive role in autoimmunity, the data on SLE are inconsistent (5). Interleukin-10 (IL-10) is secreted not only by Th2 cells but also by Treg cells. The differentiation and proliferation of Treg and Th17 cells are regulated by multiple cytokines including IL-10, IL-23, IL-17, and IL-6 (6). Regulating the balance between Treg and Th17 cells will be a promising new therapeutic target in SLE patients.

Vitamin D is an important steroid hormone that has significant effects on bone health and the cardiovascular system (7). Vitamin D also has some non-classical effects, such as immune modulatory effects (8). Many studies have found that most patients with autoimmune diseases worldwide suffer from vitamin D deficiency. These studies have also emphasized the relationship between decreased serum vitamin D levels and disease activity in SLE and rheumatoid arthritis (9–11). Vitamin D has great impact on immune cells as well as the inflammatory cascade. The receptors of Vitamin D are commonly accessible for many adaptive immune cells including T cells, B cells, macrophages, and dendritic cells (12).

Whether vitamin D can act on Treg and Th17 cells remains largely unexplored. Therefore, the purpose of this study is to investigate the relationship between Th17, Treg, cytokines, and serum 25 hydroxyvitamin D [25(OH)D] in patients with initial-onset childhood SLE.

2. Patients and methods

2.1. Study subjects

A total of 82 children aged <18 years with initial-onset SLE who were admitted to the Pediatrics Department of the Second Hospital of Hebei Medical University between April 2020 and February 2023 were included in this study. All patients met the 1997 American College of Rheumatology (ACR) classification criteria for SLE (13) or the 2012 Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) classification criteria for SLE (14). The disease activity was assessed using the SLE Disease Activity Index-2000 (SLEDAI-2K). The exclusion criteria were as follows: certain diseases that affect vitamin D metabolism (gastrointestinal surgery, liver metabolic diseases, tumors, etc.); and vitamin D supplementation by oral medication within the past 3 months. This study involved 60 healthy subjects during the same period as healthy controls (HC). This study was approved by the ethics committee of the Second Hospital of Hebei Medical University (protocol number 2021-R307).

2.2. Laboratory examinations

Laboratory examinations included routine blood tests, 24-h urine protein, erythrocyte sedimentation rate (ESR), liver function, renal function, complement 3 (C3), complement 4 (C4), antinuclear antibody, double-stranded deoxyribonucleic acid (dsDNA), serum calcium, and serum phosphorus.

2.3. Determination of serum 25(OH)D level

Blood was collected between 6:00 and 7:00 in the morning, and the children were fasted from food and water overnight before the blood samples were collected. The chemiluminescence method was performed for the detection of serum 25(OH)D levels, the kit was provided by Siemens Healthcare Diagnostics Inc. (USA), and the analysis was done using an ADVIA Centaur XP automatic chemiluminescence immunoassay analyzer. A vitamin D insufficiency was defined as serum 25(OH)D level of < 20 ng/ml, and a vitamin D sufficiency was defined as serum 25(OH)D level of ≥ 20 ng/ml.

2.4. Flow cytometry

2.4.1. Sample and cell preparations

All participants fasted from water after 12 p.m. the previous day, and peripheral venous blood samples of approximately 5 ml were collected between 6:00 and 7:00 in the morning. Blood samples were anticoagulated with ethylenediaminetetraacetic acid dipotassium (EDTA-K2), which was used to isolate and identify Treg and Th17 cell subsets. Peripheral blood mononuclear cells (PBMCs) were obtained through Ficoll density gradient. PBMCs were suspended at a density of 2×10^6 cells/ml on a complete culture medium (RPMI 1640 supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, 2 mM glutamine, and 10% heat-inactivated fetal calf serum) to obtain and analyze Th17 cell subset. The cell suspension was transferred to 24-well culture plates with a concentration of 25 ng/ml of phorbol ethyl ester (PMA), 1.7 ml of moneomycin (MN), and 1 ml of ionomycin (LC), and then incubated at 37°C under a 5% CO_2 environment for 4 h. For Treg cells analysis, PBMCs were suspended at a density of 2×10^7 cells/ml.

2.4.2. Surface and intracellular staining

To analyze Th17 cell subset, the cells were fixed and permeabilized according to the manufacturer's instructions, and then intracellularly stained with PE-conjugated anti-IL-17 monoclonal antibodies. Th17 cells were labeled as CD4 + IL-17A+. For Treg analysis, the cells were surface-stained, and then fixed permeabilized, then stained with PE anti-human Foxp3 according to the manufacturer's instructions. Treg cells were labeled as CD4 + CD25 + FoxP3+. Homotypic controls were used to verify specificity and perform compensation correction. All antibodies were provided by eBioscience. Stained cells were analyzed by flow cytometry analysis using a FACSCalibur flow cytometer (BD biosciences) with FlowJo software (Tree Star, San Carlos, CA, USA).

2.4.3. Enzyme-linked immunosorbent assay

A total of 3 ml of blood was collected and anticoagulated with EDTA-K2 to evaluate cytokines. Plasma IL-23, IL-17, TNF-α, IL-6, and IL-10 concentrations were determined by using human IL-23, IL-17, TNF-α, IL-6, and IL-10 enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Elabscience Biotechnology Co., Ltd.).

2.5. Statistical analysis

The data were statistically analyzed by using the SPSS version23.0 program. The data were presented as the mean ± standard deviation (SD) or median, and the categorical variables were expressed as frequencies and percentages. The rates were compared between two or more groups using chi-square test or Fisher's exact test. A non-parametric Mann–Whitney *U* test was used to compare the data between groups. Pearson correlation analysis was used for variables that conformed to a normal distribution, and Spearman correlation analysis was used for variables that did not conform to a normal distribution. Statistically significant was defined as *p*-value less than 0.05.

3. Result

3.1. Serum 25(OH)D level

As shown in **Figure 1**, the serum 25(OH)D levels in patients with initial-onset childhood SLE were significantly lower than those in the healthy control group (25.60 ± 6.87 ng/ml for HC, 18.86 ± 5.18 ng/ml for SLE).

3.2. Comparison of clinical and laboratory findings in pediatric SLE patients with different 25(OH)D levels

In this study, the patients with initial-onset childhood SLE were divided into two groups based on their serum 25(OH)D levels. The clinical manifestations and laboratory parameters were compared between the two groups. The proportion of lupus nephritis (LN) was higher in the vitamin D insufficiency group (71.4%) compared with the vitamin D sufficiency group (30.3%) ($p < 0.05$). SLEDAI was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$). The probability of pulmonary involvement and anemia was higher in the vitamin D insufficiency group. The SLEDAI-2K score was higher in the vitamin D insufficiency group (median = 14) than that in the vitamin D sufficiency group (median = 9) ($p < 0.05$), indicating higher disease activity in SLE. Compared with the vitamin D sufficiency group (0.54 ± .024 g/L), SLE patients in the vitamin D insufficiency group had lower levels of C3 (0.39 ± 0.27 g/L) ($p < 0.05$). It was worth noting that SLE children with insufficient vitamin D had lower serum calcium levels (**Table 1**).

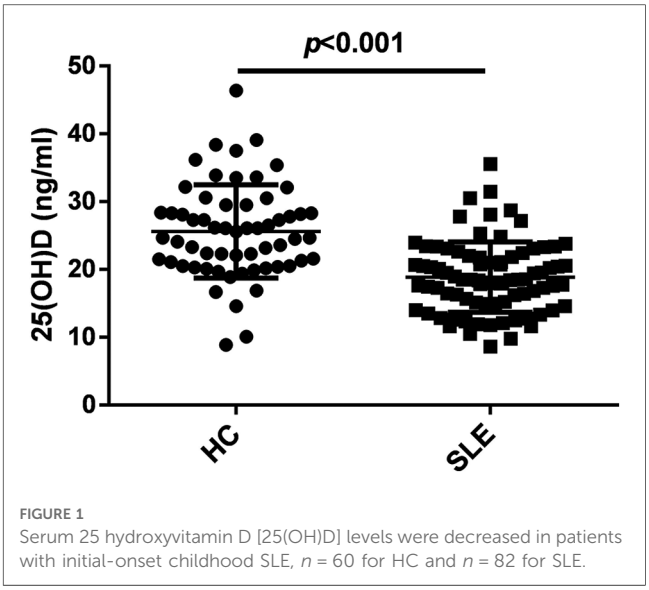

FIGURE 1
Serum 25 hydroxyvitamin D [25(OH)D] levels were decreased in patients with initial-onset childhood SLE, *n* = 60 for HC and *n* = 82 for SLE.

3.3. Correlations of 25(OH)D levels with the clinical and laboratory parameters

The 25(OH)D levels were positively correlated with C3 ($r = 0.303$, $p = 0.006$) and C4 ($r = 0.225$, $p = 0.042$), while the 25(OH)D levels were negatively correlated with 24-h urinary protein ($r = -0.423$, $p < 0.001$) (**Table 2**).

3.4. Correlations of the Treg ratio with the clinical and laboratory parameters

The Treg ratio was positively correlated with C4 ($r = 0.281$, $p = 0.011$), while it was negatively correlated with 24-h urinary protein ($r = -0.261$, $p = 0.018$) and SLEDAI ($r = -0.268$, $p = 0.015$) (**Table 3**).

3.5. Correlations of the Th17 cell ratio with the clinical and laboratory parameters

The Th17 cell ratio was positively correlated with 24-h urinary protein ($r = 0.277$, $p = 0.012$) and SLEDAI ($r = 0.287$, $p = 0.009$),

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TABLE 1 Demographic, clinical, and laboratory characteristics of the studied groups.

Characteristic	Total	25(OH)D < 20 ng/ml	25(OH)D ≥ 20 ng/ml	*p*
Female sex, n/N (%)	67/82 (81.7%)	40/49 (81.6%)	27/33 (81.8%)	0.983
Age (years), mean ± SD	11.4 ± 2.2	11.6 ± 2.4	11.2 ± 2.0	0.320
Lupus nephritis, *n* (%)	45 (54.9%)	35 (71.4%)	10 (30.3%)	**<0.001**
Arthritis, *n* (%)	22 (26.8%)	14 (28.6%)	8 (24.2%)	0.664
Mucocutaneous, *n* (%)	39 (47.6%)	23 (46.9%)	16 (48.5%)	0.891
Vasculitis, *n* (%)	1 (1.2%)	1 (2%)	0 (0%)	0.598
Serositis, *n* (%)	8 (9.8%)	7 (14.3%)	1 (3%)	0.092
Neurologic, *n* (%)	16 (19.5%)	9 (18.4%)	7 (21.2%)	0.750
Pulmonary, *n* (%)	13 (15.9%)	11 (22.4%)	2 (6.1%)	**0.046**
SLEDAI, median (range)	12 (2–33)	14 (2–33)	9 (3–31)	**0.048**
Leukopenia (<4 × 10^9/L), *n* (%)	42 (51.2%)	21 (42.9%)	21 (63.6%)	0.065
Anemia (<110 g/L), *n* (%)	46 (56.1%)	33 (67.3%)	13 (39.4%)	**0.012**
Thrombocytopenia (<100 × 10^9/L), *n* (%)	27 (32.9%)	20 (40.8%)	7 (21.2%)	0.064
Proteinuria, *n* (%)	43 (52.4%)	35 (71.4%)	8 (24.2%)	<0.001
C3 (g/L), mean ± SD	0.45 ± 0.27	0.39 ± 0.27	0.54 ± 0.24	**0.004**
C4 (g/L), mean ± SD	0.07 ± 0.06	0.07 ± 0.06	0.08 ± 0.05	0.055
Positive anti-dsDNA, *n* (%)	52 (63.4%)	33 (67.3%)	19 (57.6%)	0.368
Serum calcium (mmol/L), mean ± SD	2.17 ± 0.16	2.11 ± 0.16	2.26 ± 0.12	**<0.001**
Serum phosphorus (mmol/L), mean ± SD	1.55 ± 0.43	1.61 ± 0.47	1.46 ± 0.33	0.356

Statistically significant results are highlighted in bold.

while it was negatively correlated with C3 ($r = -0.257$, $p = 0.02$) (Table 4).

3.6. The relationship between 25(OH)D levels and the proportion of Treg and Th17 cells

As shown in Figure 2A, the Treg ratio in children with initial-onset childhood SLE decreased (5.69 ± 2.03 for HC, 2.79 ± 1.33 for SLE). The 25(OH)D levels were positively correlated with the Treg ratio ($r = 0.337$, $p = 0.002$) (Figure 2B). As shown in Figure 2C, the Th17 cell ratio in patients with initial-onset childhood SLE increased (3.52 ± 1.36 for HC, 8.16 ± 6.16 for SLE). The 25(OH)D levels were negatively correlated with the Th17 cell ratio ($r = -0.370$, $p = 0.001$) (Figure 2D).

3.7. Negative correlation between serum 25(OH)D and serum levels of cytokines

The ELISA results showed a significant increase of the levels of IL-23, IL-17, TNF-α, IL-10, and IL-6 in patients with initial-onset childhood SLE (Figures 3A–E, 9.78 ± 4.84 pg/ml vs. 27.53 ± 14.55 pg/ml; 7.80 ± 4.59 pg/ml vs. 12.77 ± 11.00 pg/ml; 3.62 ± 1.55 pg/ml vs.10.32 ± 9.57 pg/ml; 3.66 ± 1.73 pg/ml vs.6.99 ± 5.63 pg/ml; 2.72 ± 1.33 pg/ml vs.18.86 ± 15.98 pg/ml). The serum 25(OH)D levels had a negative correlation with IL-23 ($r = -0.589$, $p < 0.001$), IL-17($r = -0.351$, $p = 0.001$), TNF-α ($r = -0.283$, $p = 0.01$), IL-6 ($r = -0.392$, $p < 0.001$), and IL-10 ($r = -0.313$, $p = 0.004$) levels (Figures 3F–J).

4. Discussion

SLE is a chronic autoimmune disease distinguished by auto-antibodies development and persistent inflammation that damages multiple organs. The clinical manifestations and severity of pediatric SLE are not completely the same as those of adult SLE, with childhood SLE having more severe clinical manifestations and being more prone to involving important organs compared with adult SLE. However, research on childhood SLE has not been widely reported (12).

The increase of Th17 and the decrease of Treg subsets were reported to be the main factors related to organ damages and auto-antibodies production in SLE patients (15). The elevation of the proportion of Th17 cells with pro-inflammatory effects was reported to be positively related to the disease activity of SLE (15). Treg cells have immunosuppressive function and can induce and maintain the self-immune tolerance of the body. The

TABLE 2 Correlations of 25(OH)D levels with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	−0.423	**<0.001**
C3	0.303	**0.006**
C4	0.225	**0.042**
SLEDAI	−0.168	0.131

Statistically significant results are highlighted in bold.

TABLE 3 Correlations of the Treg ratio with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	−0.261	**0.018**
C3	0.212	0.056
C4	0.281	**0.011**
SLEDAI	−0.268	**0.015**

Statistically significant results are highlighted in bold.

TABLE 4 Correlations of the Th17 cell ratio with the clinical and laboratory parameters.

	R (Spearman correlation)	*p*
24-h urinary protein	0.277	**0.012**
C3	−0.257	**0.02**
C4	−0.155	0.164
SLEDAI	0.287	**0.009**

Statistically significant results are highlighted in bold.

decrease of Treg and its dysfunction play a very important role in the pathogenesis of SLE (1). Injecting Treg into SLE mice could alleviate inflammation and reduce tissue damage (1). Our study found that the ratio of Th17 cells significantly elevated in initial-onset childhood SLE, while the proportion of Treg significantly decreased compared with healthy controls. However, there have been reports of an increase of the percentage of Treg and Th17 cells rather than a decrease of the number of Treg in SLE patients (2). The research results on the ratio of Treg in SLE patients are inconsistent. Therefore, it is currently believed that not only abnormal proportions but, more importantly, abnormal functions of Treg are involved in the pathogenesis of SLE. Reports confirmed that the mTOR signaling pathway regulates the proliferation, differentiation, and functions of Treg cells (16). Specifically, mTORC1 promotes the expansion of pro-inflammatory lymphocyte subsets such as Th17; mTORC2 drives the proliferation of T follicle helper cells, promoting the activation of B cells and generation of auto-antibodies (17). Both mTORC1 and mTORC2 can control the differentiation and maturation of CD4 + CD25 + Foxp3 + Treg cells (17). In SLE patients, the abnormal metabolism of T cells, including high mTOR activation, increased glutaminolysis, active lipid synthesis, and enhanced glycolysis, all contribute to the differentiation and function of Th17. The metabolic disorder of T cell is a potential mechanism for Th17/Treg imbalance in SLE patients (1).

The serum 25(OH)D levels for children with SLE were obviously lower than those for healthy control children. Consistent with our findings, multiple studies worldwide have found lower levels of 25(OH)D in adults with SLE (10). Although insufficiency and/or deficiency of vitamin D have been reported in children with SLE (11, 12), there is relatively few studies on vitamin D levels in pediatric SLE. There are various reasons for the decrease of serum vitamin D levels in patients

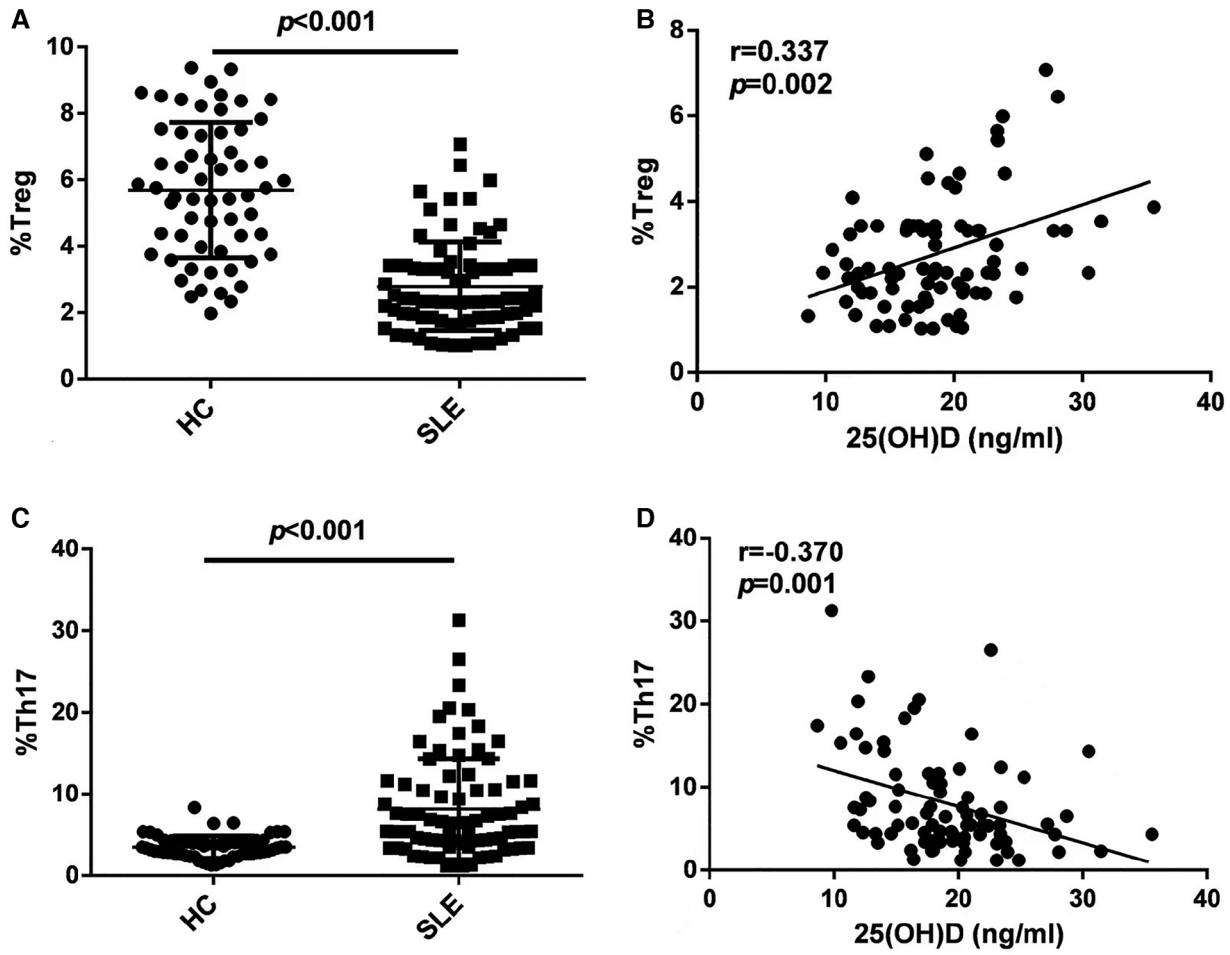

FIGURE 2
Flow cytometry analysis of Treg subset (A) and Th17 cell subset (C) in peripheral blood, *n* = 60 for HC and *n* = 82 for SLE. The correlation analysis of 25 (OH)D levels and Treg subset (B) and Th17 cell subset (D) in peripheral blood in patients with initial-onset childhood SLE (*n* = 82).

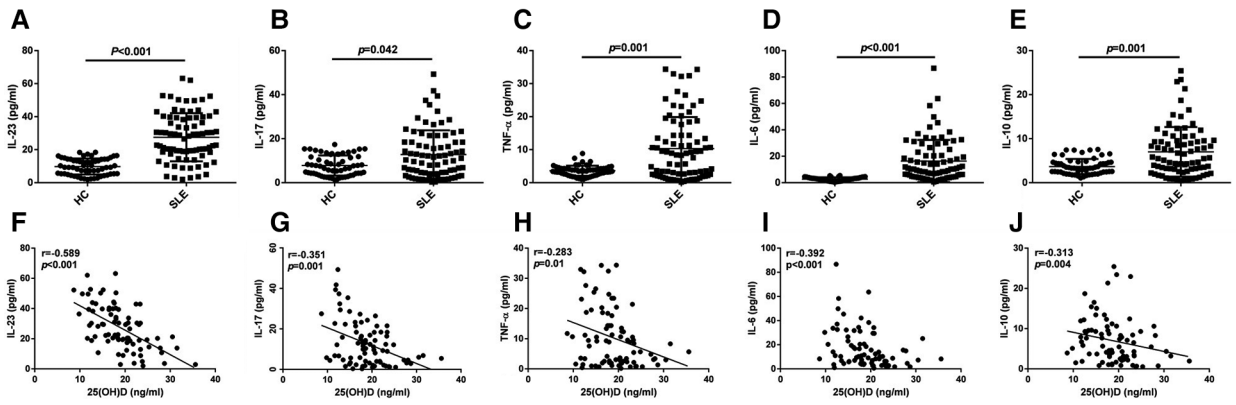

FIGURE 3
ELISA analysis of IL-23, IL-17, TNF-α, IL-6, and IL-10 (**A–E**), *n* = 60 for HC and *n* = 82 for SLE. The correlation analysis of serum 25(OH)D level and IL-23, IL-17, TNF-α, IL-6, and IL-10 in serum from patients with initial-onset childhood SLE (**F–J**) (*n* = 82).

with SLE. Vitamin D is mainly synthesized through the epidermal layer of the skin after ultraviolet exposure (7). The main measures for SLE patients to avoid photosensitivity are sunshade and using sunscreen, but these are also risk factors for vitamin D deficiency (10). Cusack et al. (18) found that using sunscreen had an impact on the levels of 25(OH)D depending on using time. Drugs used to treat SLE may exacerbate vitamin D deficiency, such as glucocorticoids reducing intestinal absorption of vitamin D and accelerating the catabolism of 25(OH)D and l,25$(OH)_2$D by enhancing 24-hydroxylase activity (19, 20). It is reported that proteinuria had a great impact on the concentration of vitamin D, which may be due to the loss of vitamin D-binding protein (DBP) caused by kidney damage in SLE (21). Our study found that the levels of serum 25(OH)D were negatively correlated with the quantification of urinary protein. Young et al. (22) found that vitamin D deficiency was driven by genetic factors, not just due to sun shielding. CYP24A1 rs4809959 modified the association of 25(OH)D and SLE. Vitamin D receptor (VDR) polymorphisms are associated with higher risk of SLE among different races, especially among Asians and Africans (23). Clinical studies found that supplementing vitamin D has an improvement effect on reducing disease activity and alleviating fatigue in patients with SLE (24).

Children with initial-onset SLE had elevated ratios of Thl7 cells and decreased ratios of Treg in their peripheral blood. The levels of 25(OH)D in patients with initial-onset childhood SLE were negatively correlated with the proportion of Th17 cells and positively correlated with the proportion of Treg cells. Th17 cell is a new CD4 + T helper cell subset discovered in recent years. Its proliferation and differentiation are different from Th1 and Th2 cells. Th17 expresses specific nuclear transcription factor rROR γT and can secrete specific cytokines such as IL-17 and IL-22 (25). Vitamin D3 signaling inhibits Th17 cell differentiation. Vitamin D3 acts on Th17 cells, inhibiting the expression of IL-22, IL-17, chemokine receptor CCR6, TNF-α, and IFN-γ, thereby preventing Th17 cells from migrating to inflammatory tissues (26, 27). The 1,25$(OH)_2$D binds the vitamin D receptor to vitamin D response element (VDRE) in the *FoxP3* gene and then directly upregulates the expression of Treg marker *FoxP3* (24). There is evidence to suggest that 1,25$(OH)_2$D can upregulate the expression of *FoxP3* in immature CD4 + T cells and induce differentiation of Treg cells, leading to an increase in the functional expression of regulatory markers such as IL-10 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (28). Research studies have also shown that Th2 and Th17 cells are transformed into plastic phenotypes through the action of 1,25 $(OH)_2$D (27). The 1,25$(OH)_2$D induces the phenotype of Treg by upregulating the expression of *FoxP3* and *CLTA4* genes, while downregulating the expression of *IL17A* genes (29).

The levels of cytokines related to Th17 cells, such as IL-6, TNF-α, IL-23, and IL-17, were significantly elevated in patients with initial-onset childhood SLE. The levels of cytokines associated to Treg cells, such as IL-10, were also elevated. The levels of IL-6, TNF-α, IL-23, IL-17, and IL-10 were negatively correlated with the serum 25(OH)D level. Research studies show that after vitamin D treatment, Treg and Th2 cells increased, and Th17 and Th1 cells decreased inconsistently (30, 31). IL-6 inhibits the expression of *Foxp3* during Treg differentiation (32). Relative studies showed that the levels of inflammatory cytokines, such as IL-6, IL-1, IL-18, and TNF-α, were significantly reduced with the vitamin D treatment group in SLE patients. On the other hand, vitamin D treatment upregulated IL-10 expression (33, 34). A study found a positive correlation between elevated serum 25 (OH)D and elevated IL-10. The author showed that after 8 weeks of vitamin D treatment, the levels of IL-10 significantly increased, while there was no significant change in the level of TGF-β1 in multiple sclerosis patients (35). However, our study showed that the levels of IL-10 increased in patients with initial-onset childhood SLE, and a negative correlation can be observed between serum vitamin D levels and blood IL-10 levels, which was inconsistent with other studies.

Vitamin D, as an immune regulatory factor, participates in innate and adaptive immunity (36). The immune regulatory role of vitamin D in autoimmune diseases has always been a focus of research (37). Multiple epidemiological studies worldwide have found vitamin D deficiency or insufficiency in various autoimmune diseases (37).

Vitamin D not only regulates Th17 and Treg cell differentiation, but also acts on other T lymphocyte subsets, B cells, dendritic cells, etc. Both T cells and B cells express VDR, which is an important target for vitamin D to exert immune regulation. Vitamin D induces tolerance phenotype by acting on antigen-presenting cell, monocyte, natural killer cell, and dendritic cells, enhance chemotaxis of neutrophil (38).

Osteoporosis can occur in patients with SLE, including juvenile patients, possibly due to chronic inflammation affecting bone metabolism and the use of glucocorticoids and other drugs (39). It is recommended to monitor the calcium and phosphorus metabolism as well as vitamin D levels in pediatric SLE patients.

Our research has limitations. The sample size included in this study is not large enough and cannot be subjected to a stratified analysis. We only studied Treg and Th17 cells and related cytokines, but did not include other lymphocyte subpopulations. We studied the relation between the serum 25 (OH)D levels and the ratio of Treg and Th17 cells in peripheral blood, but did not conduct a double-blind controlled randomized study to observe the changes in Treg and Th17 cells after vitamin D treatment. The molecular mechanism by which vitamin D acts on Treg and Th17 cells in SLE patients is not yet well understood. These will be explored in our future research.

5. Conclusion

The imbalance of Treg and Th17 cell differentiation leads to the suppression of immune function and promotes the development of SLE. In patients with initial-onset childhood SLE, the changes of serum vitamin D levels can affect the proportion of Treg cell subset and TH17 cell subset and can also affect the levels of cytokines related to these T cell subpopulations. The molecular mechanism of action of vitamin D and lymphocyte subpopulations in SLE is complex. Further exploration should be conducted on the role and mechanism of vitamin D in regulating Th17 and Treg subsets, providing a basis for immunotherapy in pediatric SLE.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Second Hospital of Hebei Medical University (protocol number 2021-R307). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

LJ performed the data analyses and wrote the manuscript. ZR contributed significantly to analysis and manuscript preparation. HZ contributed to the conception of the study. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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