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ORIGINAL ARTICLE

# Correlation between disease activity of pediatric-onset systemic lupus erythematosus and level of vitamin D in Taiwan: A case–cohort study

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vitamin D deficiency

**Abstract** *Background:* Vitamin D deficiency has been associated with systemic lupus erythematosus (SLE), but there is no consensus on the role of serum vitamin D in evaluating or predicting disease activity. This study aimed to demonstrate the direct correlation between vitamin D level and pediatric-onset SLE disease activity by a retrospective cohort study design.
*Patients and methods:* Thirty-five patients with pediatric-onset SLE and paired sera at the active and inactive disease states were enrolled. Disease activity was defined by Systemic Lupus Erythematosus Disease Activity Index 2000, and active lupus nephritis (LN) was defined as active urine sediment, and proteinuria >2+ on stick or >500 mg/day. All data were reviewed and calculated from previous medical records. The levels of both vitamin D2 and vitamin D3 were checked by electrochemiluminescence immunoassay.
*Results:* Serum 25-hydroxyvitamin D (25-OH D) levels in the active status were significantly lower compared to that in inactive disease status (12.0 ± 7.2 ng/mL vs. 15.4 ± 7.4 ng/mL, $p = 0.005$). A subgroup analysis revealed that at active disease status, patients with LN had lower 25-OH D levels than patients without LN (16.3 ± 8.2 ng/mL vs. 9.8 ± 5.6 ng/mL, $p = 0.023$). Moreover, there is a significant inverse correlation between serum 25-OH D levels and Systemic Lupus Erythematosus Disease Activity Index 2000 at both inactive ($r = -0.335$, $p = 0.003$) and active ($r = -0.373$, $p = 0.016$) disease status.
*Conclusion:* Serum vitamin D levels are inversely correlated with SLE disease activity at both active and inactive disease status, and also with the presence of LN at active disease stage.

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## Introduction

Vitamin D is a fat-soluble prohormone, and the vitamin D endocrine system classically has an important role in regulating calcium and bone homeostasis. Vitamin D is resorbed from the skin and diet, and is metabolized in the liver to 25-hydroxyvitamin D (25-OH D), which is the best marker reflecting vitamin D status. The major active form of vitamin D is 1,25-dihydroxyvitamin D. It is converted from 25-OH D in the kidneys by the enzyme 25-hydroxyvitamin D-1$\alpha$-hydroxylase. The 1,25-dihydroxyvitamin D acts on multiple targets through nuclear vitamin D receptors.[1,2]

Vitamin D deficiency is not an uncommon disorder. It affects all age and ethnic groups, and it is even more prevalent in children.[3] Currently, there are growing data regarding the association between vitamin D and immunologic or autoimmune diseases.[4–8]

Systemic lupus erythematosus (SLE) is a complicated autoimmune disease with multiple underlying immunologic mechanisms; it commonly involves the kidney, causing lupus nephritis (LN).[9] Clinical studies on vitamin D and SLE have been introduced since 1995.[10] In SLE patients, the prevalence of vitamin D deficiency ranges from 16% to 96%.[11] Most reports have been designed as cross-sectional studies with or without control groups. Despite the diverse definitions, the prevalence of vitamin D deficiency or insufficiency among SLE patients is higher than in healthy groups. Several cross-sectional studies also reveal an inverse correlation between serum vitamin D level and SLE disease activity, reinforcing the importance of vitamin D in SLE and the immune system.[12–19]

A significant number of SLE studies have been conducted over the past few years, but there is still a paucity of straight evidence of the relationship between vitamin D and pediatric-onset SLE. This study aims at providing longitudinal data to demonstrate the direct correlation between vitamin D and pediatric-onset SLE.

## Methods

In this current study, children diagnosed with SLE with both active and inactive disease status were enrolled. All demographic data, clinical manifestations, laboratory values, disease activity indices, and medications were reviewed and calculated from previous medical records (Table 1). Active disease was defined according to published definitions[20,21] and Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K).[22] Active disease was defined as SLEDAI-2K $\geq$ 5, while inactive disease was defined as SLEDAI-2K $\leq$ 4. Active LN was present in 23 patients at active disease status, and active LN was defined as active urine sediment, and proteinuria >2+ on stick or >500 mg/day. All patients were under regular outpatient department follow up, and laboratory assessment was conducted every 1–3 months based on disease severity, for an average follow-up duration of 6 years. All laboratory data were rechecked on each visit. All patients had been receiving continuous prednisolone-based treatment at both active and inactive periods, and two complete data sets for each patient were obtained at the active and inactive periods of the disease.

Laboratory tests included complete blood count with differential, creatinine, complement C3 and C4, and anti-double-strand DNA antibody. Serum and plasma samples were stored at −20°C until further use and serum 25-OH D concentrations were measured by an automated electrochemiluminescence-based assay (Elecsys Vitamin D total assay; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Vitamin D deficiency was defined as <20 ng/mL and insufficiency as <30 ng/mL, according to the published criteria.[23]

Data were analyzed by paired *t* test, Mann–Whitney *U* test, and one-way analysis of variance (SPSS Statistics 17.0.1; SPSS Inc., Chicago, United States) with a longitudinal design and with paired sera. To correct the influence

**Table 1** Demographic characteristics of patients with pediatric-onset systemic lupus erythematosus.

| | Inactive disease | Active disease | *p* |
|---|---|---|---|
| WBC ($10^3$/mL) | 5188 ± 2175 | 4774 ± 2181 | 0.242 |
| Hemoglobin (g/dL) | 12.5 ± 1.1 | 11.4 ± 2.1 | 0.458 |
| Platelet ($10^3/\mu$L) | 249.6 ± 74.9 | 185.7 ± 98.5 | 0.004 |
| Creatinine (mg/dL) | 0.55 ± 0.11 | 0.61 ± 0.23 | <0.001 |
| C3 (mg/dL) | 77.7 ± 23.0 | 59.7 ± 23.0 | 0.005 |
| C4 (mg/dL) | 12.4 ± 5.8 | 10.0 ± 6.26 | 0.019 |
| Anti-dsDNA (IU/mL) | 178.3 ± 145.6 | 261.7 ± 180.6 | <0.001 |
| SLEDAI-2K | 2.5 ± 1.7 | 12.1 ± 6.5 | 0.006 |
| Daily steroid dose (mg) | 7.9 ± 5.6 | 7.6 ± 6.1 | 0.794 |
| Cumulative steroid dose (mg) | 236.4 ± 192.7 | 255.8 ± 231.9 | 0.328 |

Data are presented as mean ± SD and analyzed with paired samples *t* test.
All study patients ($n$ = 35) had both active and inactive disease status. Laboratory test results revealed WBC count, hemoglobin, platelet, creatinine, complement C3 and C4, and anti-dsDNA. The daily and cumulative steroid use of the patients 1 month before the examination was also included in the table. All data were reviewed and calculated from previous medical records.
Anti-dsDNA = anti-double-strand DNA antibody; SD = standard deviation; SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index 2000; WBC = white blood cell.

of seasonal variability on serum vitamin D level, high-ultraviolet seasons, defined as the period from March to October, were taken into consideration. The Institutional Review Board of Chang Gung Medical Foundation approved the study (IRB: 103-2832B).

## Results

Thirty-five patients with pediatric-onset SLE and age of onset under 18 years were included. The patient's mean age at disease onset of SLE was 12.9 ± 3.12 years (range, 6–18 y). Patients who expired within 6 months from the onset of disease and those who had mixed connective tissue disorder or pre-existing major organ disease, such as irreversible renal failure, complex congenital heart disease, or chromosome anomaly, were excluded from this study. All patients went through both active and inactive disease status and had constant follow up of laboratory data every month (Table 1). Data of each patient were collected at both active and inactive disease status.

Among the 35 patients at inactive disease stage, 33 (94%) had vitamin insufficiency and 28 (80%) had vitamin D deficiency. Moreover, individual profile plots showed that compared to sera in the inactive disease status, sera in the active disease status had significantly lower serum 25-OH D levels (12.0 ± 7.2 ng/mL vs. 15.4 ± 7.4 ng/mL, $p = 0.005$; Figure 1A). The overall mean percentage change of serum 25-OH D levels of patients from inactive to active disease status was −17.9%, with the median percentage change being −30.8% (Figure 1B). A subgroup analysis also revealed that patients with LN had lower 25-OH D levels than those without LN during active disease status (16.3 ± 8.2 ng/mL vs. 9.8 ± 5.6 ng/mL, $p = 0.023$; Figure 2).

Simple linear regression was performed to demonstrate the correlation between serum 25-OH D levels and SLEDAI-2K scores. Serum 25-OH D levels in both inactive and active disease status were significantly but inversely correlated with SLEDAI-2K ($r = -0.335$, $p = 0.003$ and $r = -0.373$, $p = 0.016$, respectively; Figures 3A and 3B). In addition, univariate analysis demonstrated that serum 25-OHD status was not significantly associated with the levels of white blood cell ($p = 0.987$), hemoglobin ($p = 0.428$), platelet ($p = 0.389$), creatinine ($p = 0.775$), or anti-double-strand DNA antibody ($p = 0.243$), or daily and cumulative steroid dosages within 1 month before the examination ($p = 0.794$ and $p = 0.328$, respectively). There was also no correlation between vitamin D levels and patients' age, seasons in which serum was taken, the presence of malar rash and arthritis, or use of hydroxychloroquine.

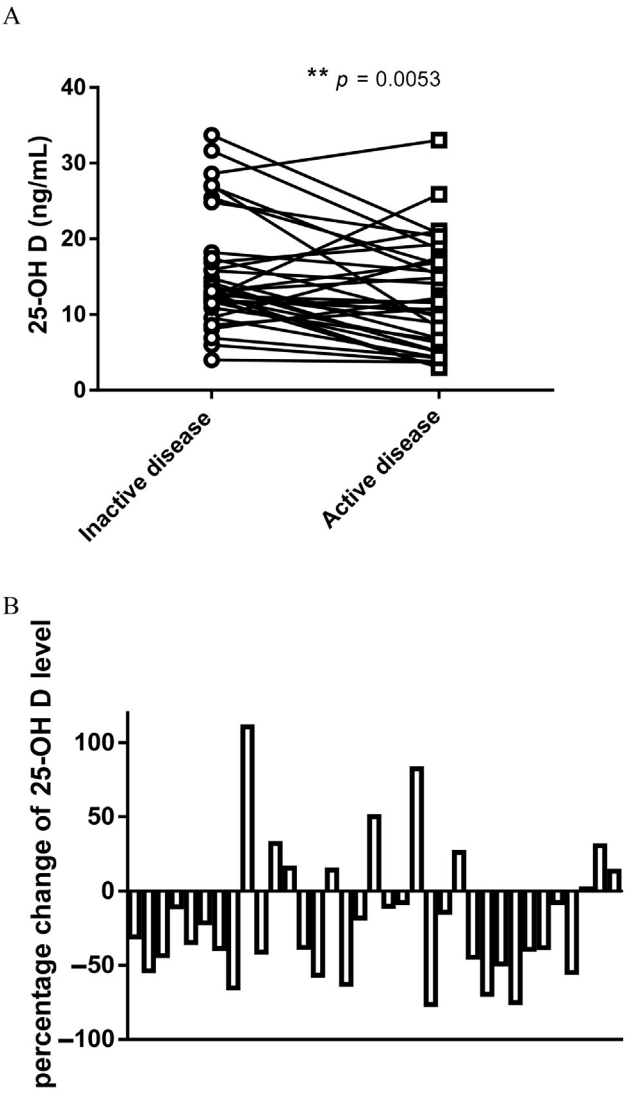


**Figure 1.** (A) Individual profile plots of 25-hydroxyvitamin D (25-OH D) levels at active and inactive disease status. Paired t test shows that the serum 25-OH D levels in active disease status is significantly lower than that in inactive disease status (12.0 ± 7.2 ng/mL vs. 15.4 ± 7.4 ng/mL, p = 0.005). (B) Percentage bar graph for changes of 25-OH D levels of individual patients from inactive to active disease status. Of the total 35 patients, 25 had lowered serum 25-OH D levels at active disease status. The mean percentage change of serum 25-OH D levels from inactive disease status to active disease status is −17.9% and the median percentage change is −30.8%. 25-OH D = 25-hydroxyvitamin D.

## Discussion

This study suggests that vitamin D deficiency and insufficiency are highly prevalent in children with SLE in Taiwan. In a previous study by Yao et al,[23] which included 1315 healthy individuals aged 10.3 ± 2.7 years in Taiwan, the prevalence of vitamin D insufficiency (25-OH D <30 ng/mL) was as high as 90.3%, while that of vitamin D deficiency (25-OH D <20 ng/mL) was 51.0%, and the mean concentration of serum 25-OH D in healthy individuals was 20.4 ± 7.1 ng/mL. Our study shows that there is even higher prevalence of vitamin D deficiency in SLE patients, regardless of their disease status (the mean concentration of 25-OH D: 15.4 ± 7.4 ng/mL for inactive disease and 12.0 ± 7.2 ng/mL for active disease).

Additionally, this study also demonstrates that at active disease status, LN can be a predictor of vitamin D deficiency. Compared to the 25-OH D levels in the inactive stage of SLE, the levels of 25-OH D in the active stage were significantly lower. Interestingly, an inverse correlation between SLEDAI-2K and serum 25-OH D levels is also

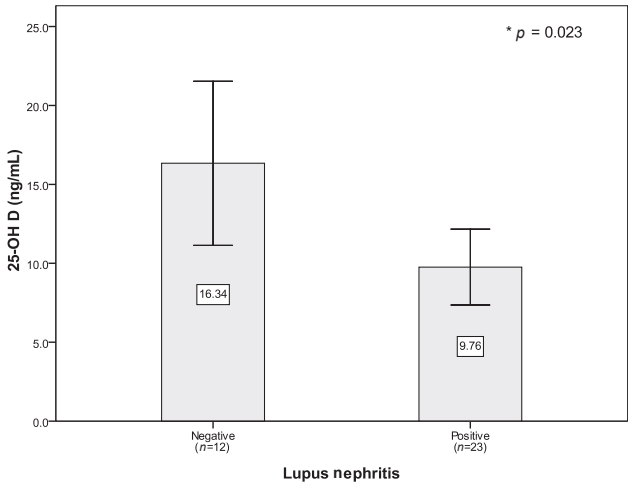


**Figure 2.** Subgroup analysis of 25-hydroxyvitamin D (25-OH D) levels in patients with and without lupus nephritis (LN) at active disease status of systemic lupus erythematosus (SLE). Two-tailed t test at 95% confidence level shows that patients with LN (n = 23) had lower 25-OH D levels than those without LN (n = 12) during active disease stage (16.3 ± 8.2 ng/mL vs. 9.8 ± 5.6 ng/mL, p = 0.023). 25-OH D = 25-hydroxyvitamin D.

observed, with the negative correlation being more significant at active disease status ($r = -0.373$ vs. $r = -0.335$). This may be explained by the possible supporting role that vitamin D has in the human immune system.[24,25] It has been suggested that immune cells such as macrophages, dendritic cells, B cells, and T cells that express the vitamin D receptor can be enhanced by sufficient vitamin D supplement.[26] Furthermore, various autoimmune diseases have been connected with vitamin D deficiency, including asthma, arthritis, Hashimoto's thyroiditis, and celiac disease. Thus, it will not be surprising if SLE also falls into this category.[27–29]

Furthermore, a study published in 2013 observed a statistically significant improvement in urine protein-to-creatinine ratio with higher levels of 25-OH D. This study reported that a 20 ng/mL increase in vitamin D was associated with a 21% decrease in the odds of having a high activity score and a 15% decrease in the odds of having clinically important proteinuria, but that there was no evidence of additional benefit beyond a serum 25-OH D level of 40 ng/mL.[30] Although the population of this study was adult SLE patients, we assume that an appropriate amount of early vitamin D supplementation may also have an immunomodulatory effect on pediatric SLE patients.

The limitations of this study include the high prevalence of vitamin D deficiency in Taiwan and the use of corticosteroids in study participants. Although the influence of high-ultraviolet seasons was corrected, it remains difficult to evaluate the possible effect that the habit of sun avoidance has on each patient, which is quite commonly seen in Asian countries including Taiwan.[31–33] Moreover, the different disease durations of each individual patient caused the intraindividual cumulative corticosteroid doses over time to differ, thereby limiting our ability to discern associations of vitamin D deficiency and disease activity.

In summary, this study shows that the serum 25-OH D levels of SLE patients in Taiwan are inversely correlated with SLEDAI-2K and that patients with LN have lower 25-OH D levels than those without LN at active disease status. These results indicate that among patients with SLE, LN may be a predictor of vitamin D deficiency. It can also be reasonably deduced from the above findings that vitamin D deficiency may play a role in the physiologic consequence

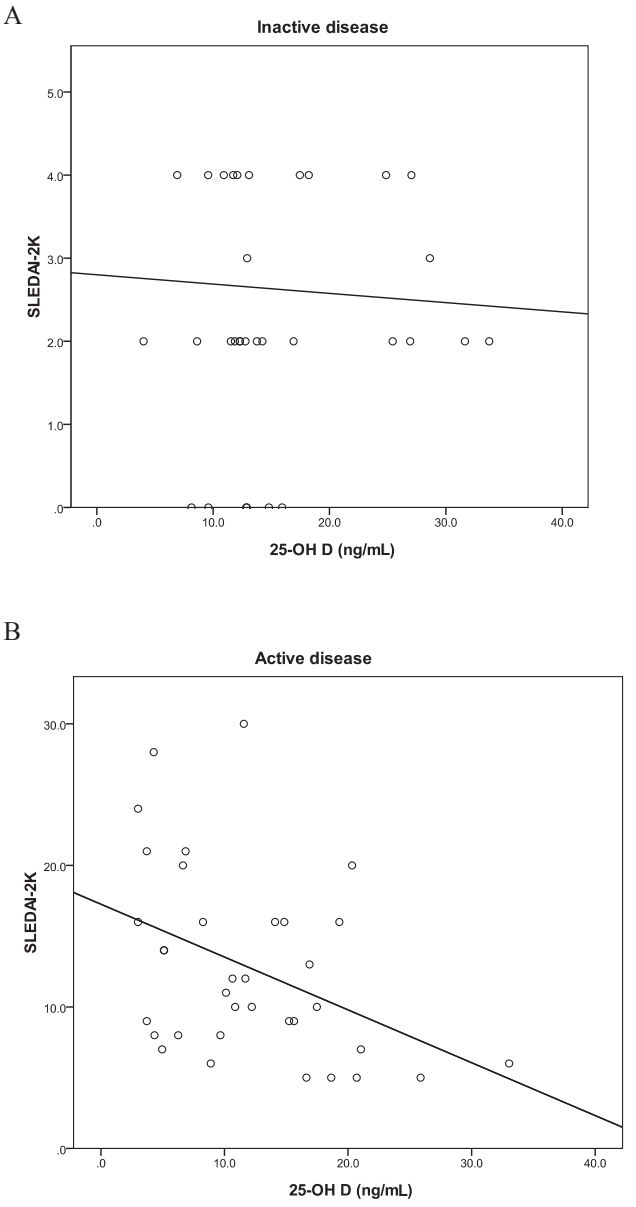


**Figure 3.** Plots of simple linear regression of 25-hydroxyvitamin D (25-OH D) levels and Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). (A) Serum 25-OH D levels were significantly but inversely correlated with SLEDAI-2K in inactive disease status ($r = -0.335$, $p = 0.003$). (B) A similar inverse correlation was observed in active disease status ($r = -0.373$, $p = 0.016$). SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index 2000; 25-OH D = 25-hydroxyvitamin D.

or the pathogenesis of SLE, and that it may be a potential factor influencing disease flare-up.

## Conflicts of interest

No financial support or other benefits from any commercial sources was received for this work.

## References


1. Plum LA, DeLuca HF. Vitamin D, disease and therapeutic opportunities. *Nat Rev Drug Discov* 2010;**9**:941—55.
2. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;**357**:266—81.
3. Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries—prevalence, risk factors and outcomes. *Nat Rev Endocrinol* 2010;**6**:550—61.
4. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. *Arch Biochem Biophys* 2000;**374**:334—8.
5. Benson AA, Toh JA, Vernon N, Jariwala SP. The role of vitamin D in the immunopathogenesis of allergic skin diseases. *Allergy* 2012;**67**:296—301.
6. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun Rev* 2011;**11**:84—7.
7. Do JE, Kwon SY, Park S, Lee ES. Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behcet's disease. *Rheumatology (Oxford)* 2008;**47**:840—8.
8. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;**358**:1500—3.
9. Borchers AT, Leibushor N, Naguwa SM, Cheema GS, Shoenfeld Y, Gershwin ME. Lupus nephritis: a critical review. *Autoimmun Rev* 2012;**12**:174—94.
10. Muller K, Kriegbaum NJ, Baslund B, Sorensen OH, 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. *Clin Rheumatol* 1995;**14**:397—400.
11. Mok CC. Vitamin D and systemic lupus erythematosus: an update. *Expert Rev Clin Immunol* 2013;**9**:453—63.
12. Casella CB, Seguro LP, Takayama L, Medeiros D, Bonfa E, Pereira RM. Juvenile onset systemic lupus erythematosus: a possible role for vitamin D in disease status and bone health. *Lupus* 2012;**21**:1335—42.
13. Souto M, Coelho A, Guo C, Mendonca L, Argolo S, Papi J, et al. Vitamin D insufficiency in Brazilian patients with SLE: prevalence, associated factors, and relationship with activity. *Lupus* 2011;**20**:1019—26.
14. Amital H, Szekanecz Z, Szucs G, Danko K, Nagy E, Csepany T, et al. 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? *Ann Rheum Dis* 2010;**69**:1155—7.
15. Ruiz E, Ramalle-Gomara E, Elena A, Quinones C, Alonso V, Posada M, et al. Trends in systemic lupus erythematosus mortality in Spain from 1981 to 2010. *Lupus* 2014;**23**:431—5.
16. Toloza SM, Cole DE, Gladman DD, Ibanez D, Urowitz MB. Vitamin D insufficiency in a large female SLE cohort. *Lupus* 2010;**19**:13—9.
17. Reynolds JA, Haque S, Berry JL, Pemberton P, Teh LS, Ho P, et al. 25-Hydroxyvitamin D deficiency is associated with increased aortic stiffness in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2012;**51**:544—51.
18. Yeap SS, Othman AZ, Zain AA, Chan SP. Vitamin D levels: its relationship to bone mineral density response and disease activity in premenopausal Malaysian systemic lupus erythematosus patients on corticosteroids. *Int J Rheum Dis* 2012;**15**:17—24.
19. Borba VZ, Vieira JG, Kasamatsu T, Radominski SC, Sato EI, Lazaretti-Castro M. Vitamin D deficiency in patients with active systemic lupus erythematosus. *Osteoporos Int* 2009;**20**:427—33.
20. Renal Disease Subcommittee of the American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. The American College of Rheumatology response criteria for proliferative and membranous renal disease in systemic lupus erythematosus clinical trials. *Arthritis Rheum* 2006;**54**:421—32.
21. Mina R, von Scheven E, Ardoin SP, Eberhard BA, Punaro M, Ilowite N, et al. Consensus treatment plans for induction therapy of newly diagnosed proliferative lupus nephritis in juvenile systemic lupus erythematosus. *Arthritis Care Res (Hoboken)* 2012;**64**:375—83.
22. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;**29**:288—91.
23. Yao TC, Tu YL, Chang SW, Tsai HJ, Gu PW, Ning HC, et al. Suboptimal vitamin D status in a population-based study of Asian children: prevalence and relation to allergic diseases and atopy. *PLoS One* 2014;**9**:e99105.
24. Bogaczewicz J, Sysa-Jedrzejowska A, Arkuszewska C, Zabek J, Kontny E, McCauliffe D, et al. Vitamin D status in systemic lupus erythematosus patients and its association with selected clinical and laboratory parameters. *Lupus* 2012;**21**:477—84.
25. Chaiamnuay S, Chailurkit LO, Narongroeknawin P, Asavatanabodee P, Laohajaroensomvat S, Chaiamnuay P. Current daily glucocorticoid use and serum creatinine levels are associated with lower 25(OH) vitamin D levels in Thai patients with systemic lupus erythematosus. *J Clin Rheumatol* 2013;**19**:121—5.
26. Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol* 2008;**4**:404—12.
27. Mackawy AM, Al-Ayed BM, Al-Rashidi BM. Vitamin D deficiency and its association with thyroid disease. *Int J Health Sci (Qassim)* 2013;**7**:267—75.
28. Comberiati P, Tsabouri S, Piacentini GL, Moser S, Minniti F, Peroni DG. Is vitamin D deficiency correlated with childhood wheezing and asthma? *Front Biosci (Elite Ed)* 2014;**6**:31—9.
29. Setty-Shah N, Maranda L, Nwosu BU. Increased risk for vitamin D deficiency in obese children with both celiac disease and type 1 diabetes. *Gastroenterol Res Pract* 2014;**2014**:561351.
30. Petri M, Bello KJ, Fang H, Magder LS. Vitamin D in systemic lupus erythematosus: modest association with disease activity and the urine protein-to-creatinine ratio. *Arthritis Rheum* 2013;**65**:1865—71.
31. Sumethkul K, Boonyaratavej S, Kitumnuaypong T, Angthararuk S, Cheewasat P, Manadee N, et al. The predictive factors of low serum 25-hydroxyvitamin D and vitamin D deficiency in patients with systemic lupus erythematosus. *Rheumatol Int* 2013;**33**:1461—7.
32. Stagi S, Cavalli L, Bertini F, Martino MD, Cerinic MM, Brandi M, et al. Vitamin D levels in children, adolescents, and young adults with juvenile-onset systemic lupus erythematosus: a cross-sectional study. *Lupus* 2014;**23**:1059—65.
33. Wahlqvist ML. Vitamin D in North-East Asian clinical nutrition practice. *Asia Pac J Clin Nutr* 2013;**22**:166—9.