Letter

Longitudinal Associations between Vitamin D Status and Systemic Inflammation Markers among Early Adolescents

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Vitamin D deficiency (VDD) has emerged as a major nutritional problem among children and adolescents. Numerous studies have documented the adverse health effects of VDD, including conditions such as chronic kidney diseases, hypoparathyroidism, and autoimmune diseases. Nevertheless, these studies were conducted only in adults and among individuals with underlying medical conditions. Adolescence is at a unique stage of growth, where nutritional deficiencies may have profound implications on their future health. Thus, there is an urgent need to conduct research on the impact of vitamin D deficiency on adolescent health.

Systemic inflammation (SI) relates to an rising risk of various physical and mental health problems, including cardiovascular diseases (CVDs), cancer, depression, autoimmune diseases, and neurodegenerative diseases. Understanding the modifiable triggers of SI could offer clues for promoting health, especially in a critical period of life, such as adolescence. Increasing evidence has shown that VDD is a notable risk factor leading to SI. Biologically, vitamin D metabolizing enzymes and vitamin D receptors are expressed in innate and adaptive immune tissues. Vitamin D plays its anti-inflammatory functions *via* a variety of mechanisms, such as upregulating MAP kinase, inhibiting the NF-κB signaling pathway, and regulating several cytokine levels[1]. A meta-analysis that reviewed eight studies conducted on children and adolescents reported a link of vitamin D levels with biomarkers of oxidative stress and inflammation in children and adolescents[2]. However, most of these studies were retrospective or cross-sectional, and only a few longitudinal cohorts have explored the relationship between vitamin D levels and inflammatory in adolescents.

Peripheral blood cell counts associated with infection and inflammation, including white blood cell (WBC) counts, lymphocyte count (LC), and neutrophil count (NC), have been reported to correlate with vitamin D levels[3]. Neutrophil-lymphocyte ratio (NLR) and platelet count (PLT) to platelet/lymphocyte ratio (PLR), derived from LC, NC, and PLT, are inexpensive biomarkers of systemic inflammation and are also known to be linked with vitamin D levels. In addition, an correlation between vitamin D levels and rising red blood cell distribution width (RDW) was reported. Associations between mean platelet volume (MPV), PLT and systemic immune inflammation index (SII) and vitamin D have also been presented in previous studies[4]. However, there is a lack of empirical research establishing an association between VDD and inflammation indexes in adolescents.

A review of previous studies found major sex differences in vitamin D deficiency, with most studies showing a higher prevalence in women than men. Previous studies have observed sexual dimorphism in inflammatory activity. Therefore, sex differences in the relationship between vitamin D and SI need to be explored. This study aimed to explore the longitudinal association and the sex differences between vitamin D levels and SI markers in early adolescence.

The study abides by the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Supplementary Table S1, available in www.besjournal.com).

Data were collected from the Chinese Early

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Adolescent Cohort (CEAC), which consisted of 1,860 early adolescents from a middle school in Huaibei City, Anhui Province, using random cluster sampling from September 2019 to September 2021. The baseline survey (wave 1) included all seventh grade students of the selected school. Demographic and health-related information of the participants was collected through questionnaires, and participants with any diagnosed organic or chronic disease (including chronic kidney and/or liver diseases and inflammatory diseases) that may impact vitamin D metabolism and inflammatory levels were excluded from the study. As blood parameters may vary according to different health conditions, adolescents with > 4 standard deviations (SDs) in the number of each cell type (WBC and RBC) and participants for whom serum 25(OH)D and blood inflammation indicator levels at wave 1 and wave 3 could not be obtained were excluded. Finally, a total of 1,423 participants were included in the final analysis (Supplementary Table S2, available in www.besjournal.com).

During the baseline and the third wave follow-up, nurses were commissioned to take fasting blood samples of the participants. After centrifuging, the serum samples were obtained and stored in the refrigerator at –80 °C until testing. The serum 25(OH)D concentrations (ng/mL) in the first and third wave were determined by LIAISON 25 OH total vitamin D assay. Using the cut-off point — 20 ng/mL for vitamin D deficiency, participants were grouped into vitamin D deficiency group (25(OH)D < 20 ng/mL) and for vitamin D non-deficiency group (25(OH)D ≥ 20 ng/mL). Vitamin D deficiency trajectories across two waves were defined as no VDD, new VDD, remitted VDD, and persistent VDD (Supplementary Table S3, available in www.besjournal.com).

At baseline and the third wave follow-up, fasting blood samples were immediately sent to the laboratory for routine blood analysis with Sysmox-XS-800i hematology analyzer. Basic measurements include WBC count, LC, NC, RDW, and PLT. PLR, NLR, and SII indicating systemic inflammation were calculated as follows:

$$PLR = PLT/LC \quad (1)$$

$$NLR = NC/LC \quad (2)$$

$$SII = PLT \times (NC/LC) \quad (3)$$

A directed acyclic graph (DAG) is used to identify confounders relating to both vitamin D levels and SI (Supplementary Figure S1, available in www.besjournal.com)[5]. The confounders included were age (used as a continuous variable), sex (male or female), residential area (urban or rural), family economic status (poor/medium or good), parents' education level (primary and secondary school or high school above), body mass index (used as a continuous variable), moderate physical activity (0, 1–2, or ≥ 3 days/week) and vigorous physical activity (0, 1–2, or ≥ 3 days/week). These factors have been found to be associated with vitamin D levels and SI.

Data analysis was conducted using SPSS (version 26.0; IBM Corp). Sample characteristics were presented based on sex. Linear regression models were performed with vitamin D level or status as the independent variable and levels of SI indexes as the dependent variable to explore the relationship between blood vitamin D levels and vitamin D deficiency with levels of inflammatory markers at baseline (n = 1,423) or 2-year follow-up (n = 1,348) after adjusting the covariates in DAG. Furthermore, sex-stratified linear regression was performed. Meta-analysis using random effects model was used to combine the associations of both time points.

Changes of vitamin D levels across two waves were calculated by using the vitamin D levels at 2-year follow-up minus vitamin D levels at baseline. Similarly, the changes of SI indexes across two waves were obtained. A set of linear regression models was performed to assess the longitudinal relationship between vitamin D levels and changes in levels of inflammation indexes across two waves after adjusting for covariates in DAG. First, VDD at baseline was used as predictors. Second, longitudinal changes in vitamin D levels were coded as predictors. Finally, longitudinal trajectories of vitamin D status were set as predictors. Moreover, sex-stratified linear regression was performed to explore whether those relationships were sex-specific. A significance level of P value 0.05 (2-tailed) was used.

Meta-analysis combining results at both time points revealed that vitamin D levels or status were negatively correlated with WBC, NC and SII, but positively correlated with MPV (as shown in Figure 1). The longitudinal analysis results further showed that: (1) Baseline VDD was significantly positively correlated with the increase of WBC, NC, RDW, PLT, PLR, NLR and SII levels; (2) Increased vitamin D levels were significantly positively correlated with MPV and negatively correlated with LC; (3) New VDD was positively correlated with LC and PLT. Persistent VDD

is positively correlated with WBC, NC, PLT, PLR, NLR and SII (Tables 1–2, Supplementary Table S4, Supplementary Figure S2, available in www.besjournal.com). All the above results indicate a significant association between serum vitamin D levels or status and levels of multiple SI markers.

WBC is a nonspecific biomarker for responding to acute inflammation; NC is generally involved in innate immune response, while LC is mainly involved in adaptive immune responses. PLT has shown the ability to recruit WBCs and release proinflammatory and anti-inflammatory factors. Despite several observational studies suggesting the potential role of vitamin D deficiency with WBC NC, LC and PLT in children and adolescents with various health conditions, there is a lack of reliable evidence derived from prospective studies. Our prospective study extended prior findings and was the first to report the longitudinal effect of vitamin D on those SI indexes in early adolescence. RDW is commonly used to differentiate the diagnosis of anemia, and can also be a inexpensive biomarker of SI. A study in the United States found serum 25(OH)D levels were inversely associated with elevated RDW[6]. In contrast, another retrospective study including 16,321 healthy children demonstrated a positive correlation between serum 25(OH)D levels and RDW[7]. Both findings lacked of longitudinal validation. However, we did not find an significant association between vitamin D levels and serum RDW levels in this study. MPV is associated with various proinflammatory diseases, and is a measure used to assess platelet reactivity, as well as platelet size and prethrombotic status. Our study show a significant positive correlation between increased vitamin D levels and MPV using longitudinal data, and the results of another study on specific dermatitis in children are consistent with ours[8]. PLR and NLR are inexpensive and readily available indicators of systemic inflammation and can be used as potential biomarkers to assess inflammation in a variety of diseases, such as cerebrovascular disease, CVD, and autoimmune diseases, and these ratios have better predictive value in assessing inflammation than lymphocytes, neutrophils, or monocytes alone. SII, as a novel inflammatory marker, is linked with an increased risk of collateral circulation development, contrast nephropathy, and CVD in previous studies. Several cross-sectional studies have shown a negative correlation between vitamin D and NLR and PLR[7,9]. Additionally, consistent with our findings, a recent study found that vitamin D status was inversely linked with SII in patients with acute coronary syndrome (ACS)[10]. Our prospective study extended prior findings and was the first to explore the effect of longitudinal vitamin D trajectory on SI in healthy adolescents.

We further conducted a sex stratification analysis based on the above analysis. The results of meta-analysis showed that Vitamin D levels of male participants were negatively associated with WBC, NC and SII, but positively correlated with MPV. No significant associations were found among female participants (Supplementary Figures S3–S6, available in www.besjournal.com). The longitudinal analysis of male subjects showed that: (1) Baseline

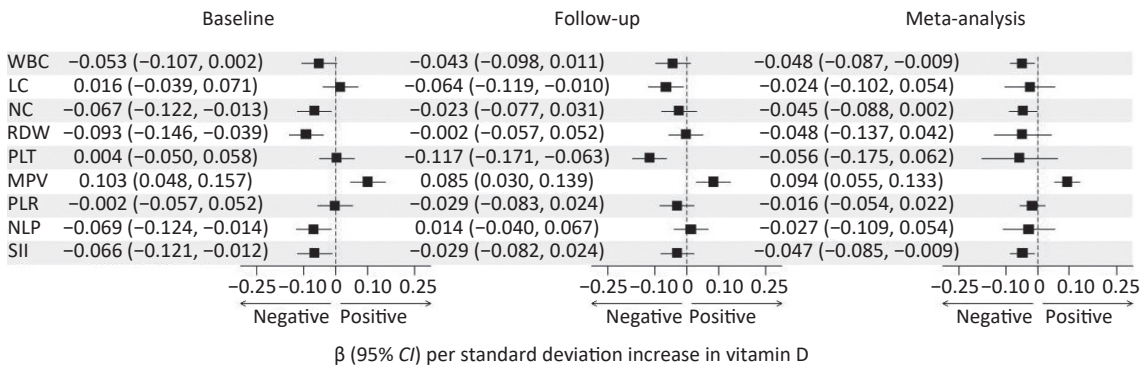

Figure 1. Linear regression analysis was performed for vitamin D levels and 9 blood markers of systemic inflammation indexes at baseline and at 2-year follow-up. Vitamin D levels and 9 blood inflammatory marker levels at both wave 1 and wave 3 was log-transformed and standardized to z-score. Meta-analysis combined results at both time points revealed significant associations between serum vitamin D levels with multiple inflammatory markers, β indicates per SD increment in vitamin D levels, β and 95% *CI* were obtained by linear regression, adjusting for age, sex, residential area, family economic status, parent's education level, BMI, moderate physical activity and vigorous physical activity.

VDD was significantly positively correlated with NC, PLT, PLR, NLR, and SII, and negatively correlated with MPV. (2) Vitamin D levels were significantly correlated with LC and MPV. (3) New VDD significantly associated with LC and PLT. Persistent VDD is positively correlated with WBC, NC, PLT, MPV and PLR, NLR and SII, and negatively correlated with MPV. Similarly, for female participants, no significant results were found in the longitudinal analysis (Supplementary Tables S5–S7, available in www.besjournal.com). In response to the observed sexual dimorphism in the association between vitamin D and SI markers, we endeavor to delve deeper into the relevant biological mechanisms in search of explanations.

Previous studies have shown that vitamin D3 can

Table 1. Correlation of vitamin D deficiency at baseline with longitudinal changes in systemic inflammation indexes

Variables	Crude model			Adjusted model		
	β	95% *CI*	*P*-value	β	95% *CI*	*P*-value
WBC	0.184	−0.094, 0.462	0.194	0.241	0.010, 0.472	0.041
LC	0.020	−0.077, 0.117	0.690	−0.020	−0.097, 0.057	0.607
NC	0.246	−0.037, 0.529	0.088	0.312	0.073, 0.551	0.011
RDW	0.021	−0.086, 0.127	0.703	0.033	−0.049, 0.115	0.432
PLT	18.748	8.889, 28.607	< 0.001	17.835	8.954, 26.715	< 0.001
MPV	0.036	−0.083, 0.154	0.557	−0.040	−0.141, 0.061	0.439
PLR	9.288	3.402, 15.174	0.002	9.355	3.884, 14.826	0.001
NLR	0.137	−0.016, 0.291	0.079	0.172	0.038, 0.307	0.012
SII	89.355	38.998, 139.712	0.001	87.587	39.883, 135.291	< 0.001

Note. Adjusted model included covariates: age, sex, residential area, family economic status, parent's education level, BMI, moderate physical activity, vigorous physical activity, and corresponding blood cell indices levels at wave. WBC, white blood cell; LC, lymphocyte count; NC, neutrophil count; RDW, red blood cell distribution width; PLT, platelet count; MPV, mean platelet volume; PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune inflammation index.

Table 2. Correlation of longitudinal changes in vitamin D levels with changes in systemic inflammation indexes

Variables	Crude model			Adjusted model		
	β	95% *CI*	*P* value	β	95% *CI*	*P* value
WBC	−0.016	−0.038, 0.006	0.158	−0.008	−0.029, 0.013	0.454
LC	−0.012	−0.019, −0.004	0.003	−0.012	−0.019, −0.005	0.001
NC	−0.003	−0.025, 0.019	0.796	0.006	−0.016, 0.027	0.610
RDW	−0.006	−0.015, 0.002	0.129	0.002	−0.006, 0.009	0.647
PLT	0.763	−0.015, 1.540	0.054	−0.519	−1.309, 0.272	0.198
MPV	0.013	0.004, 0.023	0.005	0.012	0.003, 0.021	0.009
PLR	1.020	0.559, 1.481	< 0.001	0.487	0.001, 0.974	0.050
NLR	0.011	−0.001, 0.023	0.083	0.014	−0.002, 0.026	0.051
SII	5.693	1.731, 9.654	0.005	3.360	−0.886, 7.605	0.121

Note. Adjusted model included covariates: age, sex, residential area, family economic status, parent's education level, BMI, moderate physical activity, vigorous physical activity, corresponding blood cell indices levels at wave 1, and vitamin D levels at wave 1. WBC, white blood cell; LC, lymphocyte count; NC, neutrophil count; RDW, red blood cell distribution width; PLT, platelet count; MPV, mean platelet volume; PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune inflammation index.

boost the production of testosterone by reducing the activity of aromatase, and testosterone is known to have anti-inflammatory effects, which explains the negative association between vitamin D and serum inflammatory markers in males. But multiple previous studies have suggested that the link between vitamin D and inflammation is more pronounced in women, this can be explained by the sex hormone-related mechanisms. Estrogen can reduce the expression of the *CYP24A1* gene and increase the expression of the *VDR* gene, resulting in a stronger anti-inflammatory response in women than in men. In our study, the association between vitamin D levels and inflammatory markers in the female cohort was only found to be meaningful at wave 1 and was not validated in the longitudinal cohort. This may be related to the limited sample size of this study, and we only included healthy adolescents. However, the results obtained in this study require to be verified by future study with a larger sample size.

This study has several limitations. First, compared to other studies, we did not include sufficient dietary information to assess total vitamin D3 intake, and it did not include information on the amount of daylight exposure. Second, all the included samples were only from a middle school, therefore the results are limited in generality and not nationally represented. Third, although we included some confounders, our effect estimates may have been influenced by other unmeasured or unknown confounders, such as the impact of COVID-19 and academic pressure, or other potential inflammatory-related influences. Moreover, the information about confounders was measured at baseline, the changes of the confounders were not considered. Fourth, there was no strict clinical diagnosis and screening when the subjects were included in the study. Finally, only peripheral blood cells and their related indicators were selected as inflammatory markers in this study, and it is necessary to expand research in the future to further explore the association between vitamin D and other systemic inflammatory markers.

In summary, our study revealed a negative correlation between vitamin D levels and WBC, LC, NC, PLT, NLR, PLR, SII levels, while a positive correlation was observed with MPV, with this association primarily evident among male participants. *Our findings* suggest that it is imperative to strengthen the assessment of adolescents' vitamin D level, and further conduct intervention studies targeting individuals with vitamin D deficiency among adolescents. By enhancing vitamin D levels, including increasing time spent outdoors for physical activity and administering vitamin D supplements, thus promoting the health of adolescents and reducing the risk of various diseases caused by SI.

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Authors' Contributions Ting Tang: Conceptualization, methodology, investigation, formal analysis, literature search, and writing-original draft. Xinhui Wang: Methodology, investigation, formal analysis, and writing-review & editing. Xue Wen, Min Li, and Mengyuan Yuan: Methodology, formal analysis, and writing-review & editing. Yonghan Li: Investigation, and writing-review & editing. Xiaoqin Zhong, Fangbiao Tao, and Puyu Su: Writing-review & editing. Xihua Yu: Conceptualization, methodology, and writing-review & editing. Gengfu Wang: Conceptualization, methodology, investigation, formal analysis, literature search, and writing-review & editing.

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