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Longitudinal vitamin D deficiency is inversely related to mite sensitization in early childhood

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Abstract

Background: There are few studies addressing the longitudinal analysis of vitamin D deficiency and its impact to the development of atopic diseases in early childhood.

Methods: We investigated 155 children who regularly followed up at our clinic for 5 years as subjects enrolled in a birth cohort study. The pattern of vitamin D levels from birth to 5 years of age was clustered using *K*-means method in R software. Absolute eosinophil count (AEC), total serum and specific immunoglobulin E antibodies against food (egg white, milk, and wheat) and inhalant allergens (*D. pteronyssinus*, *D. farina*, and *C. herbarum*) were measured at 1.5, 3, 4 and 5 years of age.

Results: A total of 137 children with serum samples obtained over at least 3 time-points during the follow-up period were recruited. By using *K*-means clustering, the dynamic changes in vitamin D levels was significantly stratified into 3 clusters (cluster A, ≥ 30 ng/mL, $n = 61$; cluster B, 20 - 29.9 ng/mL, $n = 53$; cluster C, < 20 ng/mL, $n = 23$). Despite no statistical association with atopic diseases, a persistent vitamin D deficiency appeared to be associated with eosinophilia at age 3, and total serum and mite-specific IgE levels at age 4. At which age, a higher prevalence of mite sensitization was significantly associated with the risk of allergic rhinitis and asthma.

Conclusions: Vitamin D deficiency is inversely associated with AEC and mite-specific IgE levels, which may potentially increase susceptibility to develop allergies including rhinitis and asthma in early childhood.

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KEYWORDS

allergic sensitization; asthma; eosinophil; longitudinal vitamin D deficiency

1 | INTRODUCTION

Vitamin D plays a key role in immune modulation and its deficiency has been proposed as a potential contributing factor in the development of allergic diseases.¹ Several studies performed in children have suggested that vitamin D deficiency may play a role in the exacerbation of asthma and influence the severity of atopic dermatitis.^{1, 2} Although a correlation has been demonstrated between vitamin D deficiency and atopic diseases, there is insufficient evidence to confirm the association between vitamin D levels and the risk of developing allergies in early childhood.

Numerous potential biomarkers, the absolute eosinophil count (AEC), total serum immunoglobulin E (IgE) levels, and allergen sensitization for atopic diseases have been widely studied.^{3, 4} Early sensitization to allergens has been identified as the most important risk factor for the development of allergic airway diseases in later life.⁴ Cross-sectional associations between vitamin D status and AEC, total IgE, and childhood asthma have been described.^{2, 5} However, it is difficult to comment on the accuracy of these associations because a single vitamin D measurement obtained in different study populations at differing ages might not provide conclusive evidence. A longitudinal analysis of the dynamic changes in vitamin D levels that influence these allergic indices and the development of atopic diseases in early childhood have not yet been fully approached.

The aim of this study was to determine the levels of serum vitamin D from birth to 5 years of age in children enrolled in a birth cohort in the Prediction of Allergies in Taiwanese Children (PATCH) study. The longitudinal changes in vitamin D levels during the follow-up period were clustered, and their correlation with allergic sensitization and impact on development of atopic disease during early childhood were assessed.

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2 | METHODS

2.1 | Patients and data collection

We enrolled children who were recruited in a birth cohort study launched at Taiwan and who completed a 5-year follow-up. Detailed descriptions pertaining to subject recruitment in this birth cohort study were reported previously.⁴ Detailed information on potential confounding variables for atopic diseases including the child's age, sex, family history of atopy, exposure to passive smoking, household income, and history of breastfeeding was collected and analyzed. The phenotypes of atopic diseases were physician-diagnosed and evaluated by the same pediatric pulmonologist at the clinic. Diagnosis of atopic diseases including eczema, allergic rhinitis, and asthma was described similar to our previous study.^{6,7} This study was approved by the Ethics Committee of Chang Gung Memorial Hospital (No. 103-6236A3). Written informed consent was obtained from parents or guardians of all study subjects.

2.2 | Measurement of serum 25(OH)D levels and clustering

Serum 25(OH)D levels were measured in cord blood at ages of 1.5, 3, 4, and 5 years using a new automated electrochemiluminescence-based assay, Elecsys Vitamin D Total assay (Roche Diagnostics, Mannheim, Germany) based on the manufacturer's instructions, as previously described.⁸ This assay has shown good precision [coefficient of variation (CV) < 13.6%], as well as excellent correlation compared to the reference method, liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS).⁹ For clustering, serum 25(OH)D levels at different ages were imported into R software (Version 3.3.1). *K*-means method was then used to group serum 25(OH)D levels into discrete and stable clusters of time series data from birth to 5 years of age.

2.3 | AEC, total serum, and allergen-specific IgE levels

A complete peripheral blood cell count was obtained using automated analysis, and the absolute eosinophil count (AEC) was calculated. As described in our previous study,⁷ total serum IgE level was measured using ImmunoCAP (Phadia, Uppsala, Sweden), while allergen-specific IgE was determined using a commercial assay for IgE (ImmunoCAP Phadiatop Infant; Phadia) for a mix of the three most common food allergens (egg white, milk, and wheat) and inhalant allergens in Taiwan (*D. pteronyssinus*, *D. farinae*, and *C. herbarum*).^{10, 11} IgE sensitization was defined as IgE levels ≥ 100 kU/L, while allergen sensitization was defined as values ≥ 0.35 kU/L.¹²

2.4 | Covariates

Confounders associated with development of atopic disease were included and adjusted using multiple logistic regression analysis. Factors including child's sex, gestational and maternal age at delivery, prenatal passive smoke exposure, maternal atopy, older siblings at birth, breastfeeding patterns, and household income were collected and analyzed.

2.5 | Statistical analysis

K-means clustering of serum 25(OH)D levels was performed using R software. Comparison of baseline characteristics and allergic sensitization categorized by serum 25(OH)D level clusters was performed using univariate parametric and nonparametric tests such as ANOVA, χ^2 , Fisher's exact test, and Kruskal-Wallis rank sum test. Multiple logistic regression analysis was used to determine the association between serum 25(OH)D level clusters and atopic diseases by adjusting for confounding factors. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Statistics for Windows Version 20.0; Armonk, NY, USA), and GraphPad Prism software (GraphPad Software Inc. Version 5.01; San Diego, CA, USA) was used to represent data graphically. All statistical hypothesis tests were two-tailed, and a p-value of < 0.05 was considered significant.

3 | RESULTS

3.1 | Study population

We initially recruited 258 children, out of which, 155 (60.1%) children who completed a 5-year follow-up at the clinic were enrolled in the study. Serum 25(OH)D levels were measured in 137 children with serum samples obtained over at least 3 time-points during the follow-up period. There were no significant differences in the baseline characteristics among these 137, 155, and all the 258 children studied.⁷ Furthermore, no significant difference in the prevalence of atopic diseases was found between children with and without blood samples obtained during the follow-up period.⁶ At 5 years of age, atopic diseases including eczema, rhinitis, and asthma were physician-diagnosed in 19, 64, and 26 of these 137 children, respectively.

3.2 | Clustering analysis of serum 25(OH)D levels

K-means clustering was performed for serum 25(OH)D levels from birth to 5 years of age using R software in 137 children. The levels of serum 25(OH)D were lowest at birth and increased sharply with a peak level at an age of 1.5 years, which then declined gradually until the age of 5 years. The dynamic changes in serum 25(OH)D levels during early childhood were stratified into three clusters significantly (Fig. 1a). Cluster A (n = 61) comprised children with serum 25(OH)D level from ≥ 30 ng/mL, at birth as well as at the age of 5 years; cluster B (n = 53) comprised with serum 25(OH)D level between 20 and 29.9 ng/mL, at birth as well as at the age of 5 years; and cluster C (n = 23) comprised children with serum 25(OH)D level from < 20 ng/mL, at birth as well as at the age of 5 years. Table 1 shows the baseline characteristics of 137 children in relation to clustering of serum 25(OH)D levels. Lower gestational age ($p = 0.046$) showed a borderline association with lower serum 25(OH)D levels, but no other significant difference was found among these three clusters. Serum 25(OH)D levels were categorized as being sufficient when ≥ 30 ng/mL (cluster A), insufficient for

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values between 20 and 29.9 ng/mL (cluster B), and deficient when < 20 ng/mL (cluster C), as described in literature.¹³

3.3 | Association of serum 25(OH)D clusters with AEC and IgE levels

Figure 1b shows the comparisons and differences between the three clusters with respect to serum 25(OH)D levels, AEC, and total serum IgE levels at different ages. A significantly higher number of eosinophils and IgE level was found in children grouped in cluster C compared to children grouped in cluster A at ages of 3 and 5 years, respectively. Using the correlation analysis, a significantly negative correlation was found between serum 25(OH)D levels and AEC at an age of 3 years (Spearman's $r = -0.367$, $p = 0.001$), and IgE levels at an age of 4 years (Spearman's $r = -0.213$, $p = 0.035$) (Fig. 2ab).

3.4 | Association between serum 25(OH)D clusters and allergic sensitization

Comparisons and differences between the three clusters with respect to serum 25(OH)D levels and allergen sensitization at different ages are shown in Fig. 1c. A significantly higher prevalence of mite sensitization was found in children grouped in cluster C compared to children grouped in cluster A at 4 years of age. Furthermore, a significantly negative correlation was found between serum 25(OH)D levels and *D. pteronyssinus*-specific IgE levels at 4 and 5 years of age (Fig. 2c). There was no significant difference in the association between serum 25(OH)D clusters and sensitization to other allergens, such as egg white, milk, and wheat.

3.5 | Association between serum 25(OH)D levels and mite sensitization and atopic diseases

Low serum 25(OH)D levels and the related high AEC and IgE levels were not associated with the risk of eczema, rhinitis, and allergic asthma throughout early childhood. However, the associated high prevalence of mite sensitization appeared to show a significantly increased risk of rhinitis [odds ratio (OR), 3.63; 95% confidence interval (CI), 1.34–9.81; $p = 0.011$] and allergic asthma (OR, 3.90; 95% CI, 1.23–12.38; $p = 0.021$) at an age of 4 years.

Several studies have been increasingly reporting a correlation between vitamin D deficiency and the incidence of allergies and asthma. However, no study describes a systematic observation and measurement of changes in vitamin D levels affecting the development of atopic diseases. This study demonstrated that a persistent vitamin D deficiency appears to be associated with eosinophilia, as well as total serum and mite-specific IgE levels. Although there is no statistical association between vitamin D levels and the risk of developing atopic disease, our results suggest that vitamin D deficiency may potentially increase eosinophil activation to produce a skewed Th2 immune response and modulate allergen sensitization to increase susceptibility to allergies including rhinitis and asthma in childhood.

Vitamin D deficiency is a global public health problem but with very limited information available in young children, particularly in terms of representative characteristics.¹⁴ In this study, the prevalence of vitamin D deficiency was noted to be low in infancy but increased gradually during early childhood, which is exactly opposite to the results obtained from a birth cohort study from Australia.¹⁵ Daily consumption of cow milk appears to be sufficient to maintain healthy vitamin D levels in most children.¹⁶ In Taiwan, milk is the primary food item until the infant is 2 years old. Despite differences among study populations and regions, this discrepancy in the distribution of vitamin D deficiency may be highly likely due to differences in feeding habits between various geographical areas.

Atopic diseases are described as a Th2 type of inflammatory disease characterized by the involvement of a specific IgE response to an allergen and with major involvement of many immune cells.¹⁷ Eosinophils are critically involved in the initiation, regulation, and maintenance of Th2 immunity.¹⁸ In this study, children with vitamin D deficiency appeared to demonstrate significantly higher AEC and serum IgE levels, supporting a previous report that vitamin D deficiency may induce eosinophilia and a skewed Th2-immune response.⁵ An inverse correlation noted between serum
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vitamin D levels and eosinophils in this study further indicates that inadequate vitamin D levels may modulate Th2 immunity partly through the up-regulation of eosinophil activation resulting in development of atopic diseases.

Several studies have linked insufficient vitamin D levels to an increased risk for allergen sensitization.¹⁹ However, not only sensitization to allergens but additionally, vitamin D levels are known to vary with age during early childhood.^{4,15} Based on the longitudinal analysis in this study, a persistent vitamin D deficiency appeared to be strongly associated with increased mite sensitization, but not with food sensitization. This finding may be because sensitization to food occurs early in life but with adequate vitamin D levels during infancy,⁴ indicating the importance of assessing their associations using longitudinal measurements.

Vitamin D deficiency is increasingly being recognized as an important risk factor in the development of pediatric allergic diseases, including eczema,²⁰ rhinitis,²¹ and asthma.²² However, these cross-sectional studies cannot provide direct evidence of changes with age and information about longitudinal development effects. In our study, a longitudinal assessment of vitamin D status demonstrated a strong inverse association between vitamin D deficiency and mite sensitization during early childhood. Exposure and sensitization to allergens have been identified as being instrumental in developing susceptibility to atopic disease and increased mite sensitization is a major risk factor for both rhinitis and asthma as in this study.⁴ Despite no direct linkage to atopic diseases, our results suggest that vitamin D deficiency may particularly modulate and increase allergen sensitization, which potentially risk the development of atopic diseases in early childhood.

A major limitation of this birth cohort study is the small sample size and short-term follow-up. A 5-year follow-up period may not represent a complete picture of changes in vitamin D levels and their relevance to the development of atopic diseases in later years. However, a major strength of this study is its short-interval follow-up and comprehensive longitudinal analysis, demonstrating valid and potentially important results.

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To conclude, our longitudinal study with measurements periodically demonstrated that vitamin D levels appear to be increased after birth with a peak at an age of 1.5 years and gradually decline thereafter, with increasing age. A persistent deficiency of vitamin D appears to be associated with eosinophilia, which may produce a skewed Th2 immune response. A significant negative correlation between serum vitamin D levels and mite-specific IgE levels further suggests that vitamin D deficiency may increase susceptibility to allergy by increasing sensitization to allergens. Although the longitudinal analyses of vitamin D are not directly related to atopic disease, vitamin D deficiency may contribute to and facilitate mite sensitization risking the development of rhinitis and asthma in early childhood.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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TABLES

TABLE 1 Baseline characteristics of 137 children in relation to vitamin D clustering from birth to the age of 5 years

	Cluster A	Cluster B	Cluster C	
Characteristics	(n = 61)	(n = 53)	(n = 23)	p-value
Family				
Maternal atopy	23 (37.7%)	28 (54.9%)	8 (34.8%)	0.120
Paternal atopy	38 (62.3%)	33 (64.7%)	9 (39.1%)	0.094
Parental smoking	30 (49.2%)	27 (51.9%)	13 (56.5%)	0.832
Older siblings	30 (49.2%)	18 (34.6%)	8 (34.8%)	0.232
Household income				0.426
Low, $\leq$ 500,000 NTD	25 (41.0%)	15 (29.4%)	9 (39.1%)	
Medium, 500,000-1,000,000 NTD	28 (45.9%)	23 (45.1%)	11 (47.8%)	
High, > 1,000,000 NTD	8 (13.1%)	13 (25.5%)	3 (13.0%)	
Infant				
Sex				0.197
Male	23 (37.7%)	28 (53.8%)	9 (39.1%)	
Female	38 (62.3%)	24 (46.2%)	14 (60.9%)	
Maternal age (yr)	31.3 $\pm$ 4.2	31.3 $\pm$ 3.7	29.0 $\pm$ 4.6	0.046
Gestational age (wk)	37.9 $\pm$ 2.0	38.5 $\pm$ 1.3	38.6 $\pm$ 1.3	0.297
Birth BMI (kg/m ²)	12.4 $\pm$ 1.8	13.1 $\pm$ 3.2	12.4 $\pm$ 0.8	0.864
Birth season				0.836
Spring (Mar. to May)	18 (29.5%)	16 (30.8%)	9 (39.1%)	

Summer (Jun to Aug.)	17 (27.9%)	16 (30.8%)	5 (21.7%)	
Fall (Sep. to Nov.)	10 (16.4%)	11 (21.2%)	3 (13.0%)	
Winter (Dec. to Feb.)	16 (26.2%)	9 (17.3%)	6 (26.1%)	
Breastfeeding				0.858
Exclusive	23 (41.1%)	22 (47.8%)	16 (47.1%)	
Partial	24 (42.9%)	17 (37.0%)	11 (32.4%)	
Formula	9 (16.1%)	7 (15.2%)	7 (20.6%)	

Data shown are mean $\pm$ s.d. or number (%) of patients as appropriate. NTD, new Taiwan dollar; yr, year; wk, week; BMI, body mass index. Mar., March; Aug., August; Sep., September; Nov., November; Dec., December; Feb., February.

FIGURE LEGENDS

FIGURE 1 Clustering of serum 25(OH)D levels during early childhood and their relationships between AEC, IgE levels and allergic sensitization at different years of age. The pattern of serum 25(OH)D levels from birth to age 5 clustering by using *K*-means method in R software (a). Comparisons and differences of serum 25(OH)D clustering with AEC, IgE levels (b) and allergen sensitization to mite, food and IgE sensitization (≥ 100 kU/L) (c) at different years of age. The box-plot showing the median and the 10th, 25th, 75th and 90th percentile. p-values referred to the comparisons of AEC at age 3, IgE levels at age 5 and mite sensitization at age 4 [cluster C vs. cluster A] are indicated by the marker. * $p < 0.05$.

FIGURE 2 Correlation of serum 25(OH)D levels with AEC, total serum IgE and *D. pteronyssinus*-specific IgE levels. Significant correlations were observed between serum 25(OH)D levels and AEC at age 3 (a), and between total serum IgE levels at age 4 (b), and between *D. pteronyssinus*-specific IgE levels at age 4 and 5 (c) respectively.



