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Correlation Between Serum Vitamin D Level and the Severity of Atopic Dermatitis Associated With Food Sensitization

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Purpose: A growing body of literature has linked vitamin D deficiency with allergic diseases, particularly atopic dermatitis (AD). In this study, we investigated the association between serum vitamin D status and the clinical manifestation of AD. We also developed an analytical method for the simultaneous determination of 25-hydroxy vitamin D_3 (25(OH)D_3), using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS). **Methods:** This study included 157 patients (79 males and 78 females) with AD, aged 4 months to 56 years. We evaluated disease severity using the SCORing Atopic Dermatitis (SCORAD) index. Serum levels of 25(OH)D_3 were determined by LC coupled with MS/MS. Total IgE and specific IgE levels were assayed using the immunoCAP system. ANOVA was used for statistical evaluation. **Results:** We found mild, moderate, and severe AD in 30 (11.1%), 87 (55.4%), and 40 (25.5%) patients, respectively. There was no significant correlation between serum levels of 25(OH)D_3 and AD severity. However, among the 36 patients with food sensitization, the mean±SD serum levels of 25(OH)D_3 were significantly higher ($P<0.05$) in patients with mild disease (21.2±5.18 ng/mL) compared with the levels in patients with moderate (17.9±4.02 ng/mL) or severe AD (13.3±5.11 ng/mL) disease. **Conclusions:** These results suggest that vitamin D deficiency is related to the severity of AD associated with food sensitization. Thus, these data suggest a role for vitamin D in a select group of AD patients.

Key Words: Atopic dermatitis; vitamin D; food allergy

INTRODUCTION

An increasing incidence of atopic diseases, including AD, has been recorded in many countries in the last 10 years. This rapid increase has most likely resulted from environmental changes, with consequential changes in gene–environment interactions. In the UK, researchers have noted that the increase in asthma and atopy was preceded and paralleled by dietary changes in society. Moreover, a decrease in vegetable consumption, particularly potatoes and green vegetables, has been observed.[1] These findings suggest that a Westernized diet deficient in antioxidants increases susceptibility to atopic diseases. Another study reported regional differences in the number of prescriptions of EpiPens within the United States; the highest rates were found in New England, and the lowest in the Southern states.[2] These results may provide important etiologic clues and indicate the potential role of vitamin D in atopic disease susceptibility. Ehlayel et al.[3] showed, in a case-controlled study, that lower serum levels of 25(OH)D_3 in children were associated with more allergic disease and elevated serum IgE levels.

There has also been increasing awareness of the importance of one potential factor–vitamin D–in the development and progress of atopic diseases. A growing body of literature has linked decreased serum vitamin D levels with allergic diseases, particularly AD.[2-4] However, large-scale prospective and randomized studies are lacking. Thus, the available data highlights the need for further analysis of the associations between vitamin D and atopic diseases in different populations.

In this study, we investigated the association between serum vitamin D status and the clinical manifestation of AD. We also developed an analytical method for the simultaneous determination of 25(OH)D_3, using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS).

MATERIALS AND METHODS

Subjects

This study included 157 patients (79 males and 78 females),

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with a mean age of 9.92 years (range, 4 months to 56 years), who were diagnosed with AD by an allergy specialist at our clinic according to the diagnostic criteria proposed by Hanifin and Rajka.[5] Patients were recruited between March 2008 and March 2012 at the Atopy Asthma Center, Seoul Medical Center, Korea. Using the SCORAD index, we scored each patient as having mild (<15), moderate (15-40), or severe (>40) disease.

This study was approved by the institutional review board of the Research Institution, Seoul Medical Center.

Laboratory tests

Levels of total IgE and IgE specific for causative food allergens were measured using ImmunoCAP (Pharmacia, Uppsala, Sweden). A specific IgE level >0.7 kU_A/L was used to define sensitization to egg white, milk, soybean, peanuts, wheat, or fish.

Method

For analysis of vitamin D in serum, we used liquid-liquid extraction (LLE) and solid phase extraction (SPE). We added 200 μL of isopropanol to 100 μL of serum, which was spiked with internal standards at a concentration of 100 ng/mL; mixtures were vortexed for 5 minutes, sonicated for 10 minutes, and centrifuged at 15,500×g for 5 minutes. Supernatants were then transferred to a new centrifuge tube.

For the SPE experiments, the SPE plates were activated with 200 μL of methanol and 200 μL of 60% methanol. The samples were then loaded onto the SPE plates and extracted. Plates were first washed with 5% methanol followed by washing with 60% methanol. Samples were then eluted with 80 μL of 5% isopropyl in methanol, followed by 50 μL of deionized water, into the plate. The mixture was then injected into the LC-MS/MS system for analysis.

Statistical analysis

The vitamin D levels among AD severity levels according to the SCORAD index were compared by ANOVA. A value of $P<0.05$ was considered to indicate statistical significance. Data analysis was conducted using STATA, version 10.0 (StataCorp, College Station, TX, USA).

RESULTS

We found mild, moderate, and severe AD in 30 (11.1%), 87 (55.4%), and 40 (25.5%) patients, respectively.

Mean±SD total serum IgE levels were higher in patients with severe disease (553.63±771.387 IU) than those with moderate (387.90±568.755 IU) or mild disease (241.61±318.766 IU) (Table 1). But that was not statistically significant.

The serum concentrations of $25(OH)D_3$ were found to be sufficient, insufficient (20-29 ng/mL) and deficient (<20 ng/mL),

Table 1. Clinical characteristics of the subjects

Severity of atopic dermatitis	Mild	Moderate	Severe	*P* value
N (%)	30 (11.1)	87 (55.4)	40 (25.5)	0.670
Age (yr)	9.59±13.73	8.38±10.44	13.55±11.05	0.058
Vit D (ng/mL)	15.59±5.97	15.52±5.61	14.66±5.67	0.701
Total eosinophil count	461.90±351.16	746.16±1511.88	510.00±287.07	0.459
Total IgE (IU)	241.61±318.77	387.90±568.76	553.63±771.39	0.187

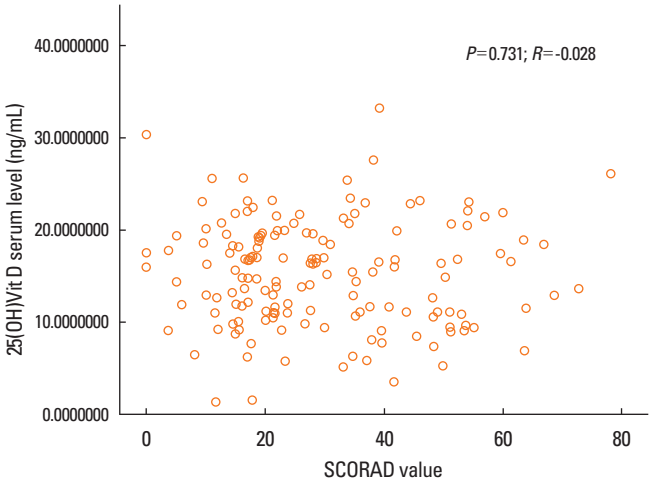

Fig. 1. Correlation between serum $25(OH)D_3$ levels and individual atopic dermatitis disease severity.

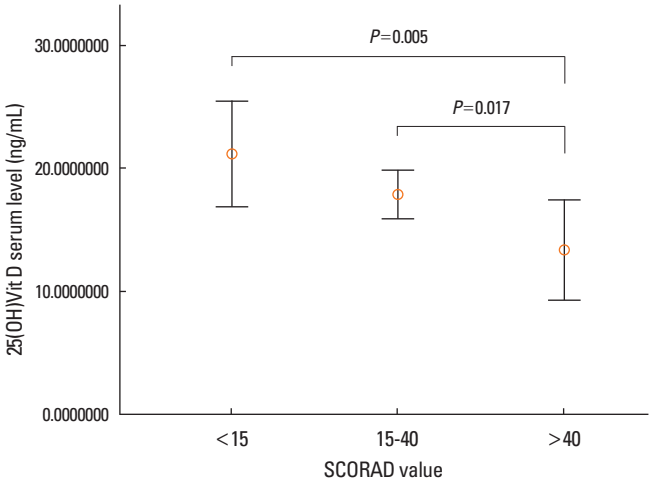

Fig. 2. Serum $25(OH)D_3$ levels according to atopic dermatitis severity associated with food sensitization. Circles indicate mean values.

Table 2. Serum 25(OH)D_3 levels according to age

Age (yr)	n (%)	Serum 25(OH)D_3 (mean±SD)
0-5	86 (54.8)	15.73±5.27
6-15	29 (18.5)	14.51±5.79
15-	42 (26.7)	15.01±6.40
Total	157	15.31±5.67

respectively, in two (1.2%), 29 (18.5%), and 126 patients (80.3%).

There was no significant correlation between serum levels of 25(OH)D_3 and AD severity (Fig. 1). However, among the 36 patients with food sensitization, mean±SD serum levels of 25(OH)D_3 were significantly higher ($P<0.05$) in patients with mild disease (21.2±5.18 ng/mL) compared to those with moderate (17.9±4.02 ng/mL) or severe AD (13.3±5.11 ng/mL) (Fig. 2).

There was no difference in the serum levels of 25(OH)D_3 according to age (Table 2).

DISCUSSION

According to the results of the 2005–2006 National Health and Nutrition Examinations Survey (NHANES), vitamin D deficiency is associated with higher levels of IgE sensitization in children and adolescents.[6] To assess the relationship between vitamin D levels and respiratory outcomes, other analyses were performed on the same survey data. A protective effect for vitamin D against wheezing and asthma was found to be strongest for non-atopic and older subjects, suggesting that vitamin D modifies the risk of allergic and respiratory disease through multiple mechanisms.[7]

Although vitamin D deficiency is defined as a serum level <20 ng/mL of 25(OH)D_3, there is also an increasing concern about vitamin D insufficiency, which is characterized by levels of 25(OH)D_3 between 20 and 29 ng/mL.[8] In this study, we found that most participants with AD could be categorized as having vitamin D insufficiency. Although there was no healthy control group, our findings showed lower normal levels of serum vitamin D (15.61±5.67 ng/mL) irrespective of AD severity, which suggests a correlation between AD and the absorption and metabolism of vitamin D.

This metabolism begins with two processes: absorption into the skin as vitamin D_3 and via the gut as either vitamin D_2 or vitamin D_3. These molecules are then metabolized in the liver to 25(OH) vitamin D, the vitamin D pro-hormone, which is usually used to measure vitamin D levels clinically. This molecule is subsequently metabolized in the kidney to its active form, 1,25$(OH)_2D_3$, which plays a key role in skeletal and extraskeletal functions, including immunity and glucose metabolism.[8] Since it is absorbed via the skin, the impact of vitamin D levels on allergic skin diseases is of particular interest.

Therefore, the relationship between vitamin D and AD has been investigated both here and in previous studies. There are several biologically plausible explanations for the inverse correlation between serum vitamin D and the presence of AD. For example, vitamin D impacts the innate immune system by stimulating the production of cathelicidin, which is an anti-microbial peptide activated through toll-like receptors (TLRs). The 1,25$(OH)_2D_3$ molecule induces antimicrobial peptide gene expression in isolated human keratinocytes, monocytes, neutrophils, and human cell lines.[9] TLR activation of human macrophages up-regulates the expression of the vitamin D receptor and vitamin D-1-hydroxylase genes, leading to the induction of the cathelicidin.[10] These data suggest a link between TLRs and vitamin D-mediated innate immunity, indicating that a difference in the ability of human populations to produce vitamin D may contributes to susceptibility to microbial infection. Among the 36 patients with food sensitization who showed a significant correlation between serum levels of 25(OH)D_3 and the severity of AD, three patients (8.3%) with impetigo were treated with antibiotics.

Based on the relationship between vitamin D deficiency and AD, trials of vitamin D supplementation in atopic populations or high-risk groups have been conducted. Supplementation with oral vitamin D in 14 atopic subjects with moderate-to-severe atopic dermatitis for 21 days resulted in a significant increase in cathelicidin expression in AD-lesional skin.[11] Another pilot study of winter-time onset or exacerbation of AD also showed a favorable impact of vitamin D supplementation.[12] However, there are conflicting data surrounding the effect of vitamin D on the development of allergic skin disease. Camargo et al.[13] found no decreased risk of AD in the children of mothers with higher intakes of vitamin D. A Northern Finland birth cohort indicated the association between vitamin D supplementation in the first year of life and an increased risk of atopy at age 31 years.[14] A further prospective study reported that an increased concentration of 25(OH)D_3 in maternal serum predisposed infants to AD at 9 months of age.[15] These variable results may be secondary to the differences in the absolute amount of vitamin D exposure, the baseline vitamin D status, and the timing of exposure.

To date, most reports on this topic have been case-controlled or cross-sectional studies, and have not ascertained any causal relationships between vitamin D deficiency and AD. However, recent studies have employed advanced protocols. Chi et al.[16] assessed the influence of prenatal vitamin D status on immune function at birth. They found that a higher umbilical cord plasma concentration of 25(OH)D_3 was associated with a lower number of T-regulatory cells, which suggests that vitamin D *in utero* may influence immune regulation in early life. Another prospective birth cohort study investigated vitamin D deficiency and food sensitization, and genotyped 11 genes involved in regulating IgE and 25(OH)D_3 concentration. In that study, a significant interaction between IL4 gene polymorphism and vitamin D deficiency was observed.[17] These data indicate that vita-

min D deficiency may increase the risk of food sensitization in certain genotypes, providing evidence of gene–vitamin D interaction in food sensitization. Our results showing an inverse correlation between serum levels of 25(OH)D_3 and AD severity in the food-sensitized group support this interpretation. According to another point of view, strict restriction of the offending food allergen provides an alternative explanation for vitamin D deficiency. Moreover, the mean age of the food-sensitized group was 6.78 years, which was relatively younger than that of the entire subject population, which averaged 9.92 years. Individuals at this younger age could have limited outdoor activity, resulting in less sun exposure. Vassallo et al.[18] also showed that food allergy is more common in those individuals who were born in the fall and winter seasons. They proposed that these differences are mediated by seasonal differences in ultraviolet light B. As our study is limited by its retrospective cross-sectional design, we could not analyze the relationship between sun exposure and vitamin D concentration. Based on the identification of vitamin D receptors in relevant immune cells, it has been suggested that the vitamin D pathway plays a role in asthma and atopy.[19,20] Using single nucleotide polymorphism selection and genotyping, Poon et al.[20] found six variants that are strongly associated with asthma and four with atopy. These efforts to clarify the relationship between vitamin D and AD could lead to the identification of potential targets for therapeutic intervention in AD.

In conclusion, the results of this study suggest that vitamin D deficiency might be related to the severity of AD that is associated with food sensitization. Thus, these data indicate the potential role of vitamin D in a select group of AD patients.

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