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

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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 indications of EpiPens within the United States; the highest rates were found in New England, and the lowest in the Southern states.

MATERIALS AND METHODS

Subjects

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

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* There are no financial or other issues that might lead to conflict of interest.
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. 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/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 ± 771.39 IU) than those with moderate (387.90 ± 568.76 IU) or mild disease (241.61 ± 318.77 IU) (Table 1). But that was not statistically significant.

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

**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 levels and individual atopic dermatitis disease severity.

**Fig. 2.** Serum 25(OH)D levels according to atopic dermatitis severity associated with food sensitization. Circles indicate mean values.
respectively, in two (1.2%), 29 (18.5%), and 126 patients (80.3%).

There was no significant correlation between serum levels of 25(OH)D₃ and AD severity (Fig. 1). However, among the 36 patients with food sensitization, mean ± SD serum levels of 25(OH)D₃ 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)D₃ 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.⁵ 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)D₃, there is also an increasing concern about vitamin D insufficiency, which is characterized by levels of 25(OH)D₃ 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 D₃ and via the gut as vitamin D₂ or vitamin D₃. 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)₂D₃, which plays a key role in skeletal and extraskeletal functions, including immunity and glucose metabolism.⁹ 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)₂D₃ molecule induces antimicrobial peptide gene expression in isolated human keratinocytes, monocytes, neutrophils, and human cell lines.¹⁰ 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.¹¹ 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 contribute to susceptibility to microbial infection. Among the 36 patients with food sensitization who showed a significant correlation between serum levels of 25(OH)D₃ 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.¹² Another pilot study of winter-time onset or exacerbation of AD also showed a favorable impact of vitamin D supplementation.¹³ However, there are conflicting data surrounding the effect of vitamin D on the development of allergic skin disease. Camargo et al.¹⁴ 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.¹⁵ A further prospective study reported that an increased concentration of 25(OH)D₃ in maternal serum predisposed infants to AD at 9 months of age.¹⁶ 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.¹⁷ 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)D₃ 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)D₃ concentration. In that study, a significant interaction between IL4 gene polymorphism and vitamin D deficiency was observed.¹⁸ These data indicate that vita-
vitamin 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)D3 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.

ACKNOWLEDGMENTS

This study was supported by grants from the Seoul Medical Center Research Institute 2011.

REFERENCES

1. Department for Environment, Food and Rural Affairs. Consumption of selected household foods (GB) 1942 to 2000 [Internet]. London: HMSO [accessed 2004 Jan 11]. Available from: www.defra.gov.uk/statistics/files/defra-stats-family-food-nfs-allfood.xls.
2. Camargo CA Jr, Clark S, Kaplan MS, Lieberman P, Wood RA. Regional differences in EpiPen prescriptions in the United States: the potential role of vitamin D. J Allergy Clin Immunol 2007;120:131-6.
3. Ehlayel MS, Bener A, Sabbah A. Is high prevalence of vitamin D deficiency evidence for asthma and allergy risks? Eur Ann Allergy Clin Immunol 2011;43:81-8.
4. 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.
5. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol Suppl (Stockh) 1980;92:44-7.
6. Sharief S, Jariwala S, Kumar J, Muntrer P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 2011;127:1195-202.
7. Keet CA, McCormack MC, Peng RD, Matsui EC. Age- and atopy-dependent effects of vitamin D on wheeze and asthma. J Allergy Clin Immunol 2011;128:414-6.e5.
8. Greer FR. Defining vitamin D deficiency in children: beyond 25-OH vitamin D serum concentrations. Pediatrics 2009;124:1471-3.
9. Wang TT, Nestel FP, Bourdoue V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.
10. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubler J, Wu K, Meikin C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zigel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770-3.
11. Hata TR, Kotol P, Jackson M, Nguyen M, Paik A, Udall D, Kanaka K, Yamasaki K, Alexandrescu D, Gallo RL. Administration of oral vitamin D induces cathelicidin production in atopic individuals. J Allergy Clin Immunol 2008;122:829-31.
12. Sidbury R, Sullivan AE, Thadhani RJ, Camargo CA Jr. Randomized controlled trial of vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. Br J Dermatol 2008;159:245-7.
13. Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 2007;85:788-95.
14. Hyyppänen E, Sovio U, WJST M, Patel S, Pekkanen J, Hartikainen AL, Jarvelin MR. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004;1037:84-95.
15. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C; Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008;62:68-77.
16. Chi A, Wildfire J, McLoughlin R, Wood RA, Bloomberg GR, Kattan M, Gergen P, Gold DR, Witter F, Chen T, Holick M, Visness C, Gern J, O’Connor GT. Umbilical cord plasma 25-hydroxyvitamin D concentration and immune function at birth: the urban environment and childhood asthma study. Clin Exp Allergy 2011;41:842-50.
17. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S, Arguelles L, Ku JY, Järvelinb MR. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004;1037:84-95.
18. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.
19. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D3 receptor in the immune system. Arch Biochem Biophys 2000;374:334-8.
20. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.
21. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S, Arguelles L, Ku JY, Järvelinb MR. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004;1037:84-95.
22. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C; Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008;62:68-77.
23. Chi A, Wildfire J, McLoughlin R, Wood RA, Bloomberg GR, Kattan M, Gergen P, Gold DR, Witter F, Chen T, Holick M, Visness C, Gern J, O’Connor GT. Umbilical cord plasma 25-hydroxyvitamin D concentration and immune function at birth: the urban environment and childhood asthma study. Clin Exp Allergy 2011;41:842-50.
24. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S, Arguelles L, Ku JY, Järvelinb MR. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004;1037:84-95.
25. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D3 receptor in the immune system. Arch Biochem Biophys 2000;374:334-8.
26. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.
27. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S, Arguelles L, Ku JY, Järvelinb MR. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 2004;1037:84-95.
28. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D3 receptor in the immune system. Arch Biochem Biophys 2000;374:334-8.
29. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.
30. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Mendoza L, Lin R, Hanahan JW, Mader S, White GH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909-12.