

ORIGINAL ARTICLE

Vitamin D deficiency is associated with diagnosis and severity of childhood atopic dermatitis

Susan Shuxin Wang¹, Kam Lun Hon¹, Alice Pik-shan Kong², Henry Nga-hin Pong¹, Gary Wing-kin Wong¹ & Ting Fan Leung¹

¹Department of Pediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong; ²Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

To cite this article: Wang SS, Hon KL, Kong AP-s, Pong HN-h, Wong GW-k, Leung TF. Vitamin D deficiency is associated with diagnosis and severity of childhood atopic dermatitis. *Pediatr Allergy Immunol* 2013; **00**.

Keywords

atopic dermatitis; atopy; children; eczema; severity; vitamin D

Correspondence

Ting Fan Leung, Department of Pediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong, 6/F, Lui Che Woo Clinical Sciences Building, Prince of Wales Hospital, Shatin, NT, Hong Kong.
Tel.: (852) 2632 2981
Fax: (852) 2636 0020
E-mail: tleung@cuhk.edu.hk

Accepted for publication 7 October 2013

DOI:10.1111/pai.12167

Abstract

Background: Recent studies implicated the importance of vitamin D in innate immune defense and pathogenesis of allergic diseases. However, the impact of vitamin D deficiency on atopic dermatitis (AD) diagnosis and severity remains unclear. This case-control study investigated such relationship in Hong Kong Chinese children.

Methods: Serum 25-hydroxyvitamin D [25(OH)D] levels of 498 AD children and 328 non-allergic controls were measured by immunoassay. Subjects were categorized into deficient (<25 nm), insufficient (25–49.9 nm), and sufficient (≥50 nm) groups. Short-term and long-term AD severity was evaluated by physician-diagnosed SCORing Atopic Dermatitis (SCORAD) and Nottingham Eczema Severity Score (NESS), respectively. Atopy biomarkers were also measured for analysis.

Results: The mean (s.d.) serum 25(OH)D levels in AD patients and controls were 28.9 (15.3) and 34.2 (14.5) nm, respectively ($p < 0.001$). More patients had serum 25(OH)D levels <25 nm than controls (47.8% vs. 26.6%). AD severity as indicated by both SCORAD and NESS showed inverse associations with serum 25(OH)D levels (respective $p = 3.6 \times 10^{-4}$ and 0.004 when adjusted for age, sex, month of assessment, and immunoassay batch as covariates). Vitamin D-deficient patients (3.08 ± 0.76) had higher logarithm-transformed total IgE than those with insufficient (2.74 ± 0.69) and sufficient (2.72 ± 0.72) serum 25(OH)D levels ($p < 0.001$). The proportion of subjects with elevated IgE was higher in vitamin D-deficient (43.2%) than vitamin D-sufficient (20.0%) groups.

Conclusions: Vitamin D deficiency and insufficiency are prevalent in Hong Kong Chinese children. Vitamin D deficiency is associated with childhood AD and high total IgE. Serum 25(OH)D levels correlate inversely with both long- and short-term AD severity.

Vitamin D is well known to be an important nutrient to bone health, which also serves as a hormone involved in obesity, cancer, cardiovascular diseases, immune function, and maternal/fetal health (1). Besides, vitamin D has emerged as a new contributor to immune regulations as well as the outcome of immune responses (2, 3). The protective role of vitamin D against allergy was supported by the associations between low serum 25-hydroxyvitamin D [25(OH)D] levels and higher rates of asthma, food allergy, and IgE sensitization (4). However, other reports suggested high vitamin D intake to be associated with increased prevalence of atopic manifestations in children. Oral supplement with cod liver oil increased risk of asthma and hay fever, and high maternal serum vitamin D during pregnancy increased the risk of infant eczema at 9 months. Studies at cellular and molecular level reported that the active

form of vitamin D $1\alpha,25(\text{OH})_2\text{D}$ increased cathelicidin in macrophages, lead to skew to T helper 2 response in T cells, as well as reduce maturation and migration of dendritic cells, lessen IgE production in B cells (5).

Atopic dermatitis (AD) is a common chronic allergic disease which prevalence is estimated to be 15–30% in children and 2–10% in adults (6). The incidence of eczema is higher in urban areas and in families with higher socioeconomic status. The prevalence of eczema ever was 19.3% in Hong Kong school-children (7). Regarding the impact of vitamin D to eczema, a small study of 37 eczema children found significant correlation between serum 25(OH)D levels and AD severity (8). In contrast, Allen et al. (9) did not find significant association between eczema and low serum 25(OH)D levels among 577 Australian infants who participated in the population-based

HealthNuts study. Nonetheless, those with vitamin D insufficiency were more likely to be peanut and/or egg allergic. Prospective studies reported contradictory findings on this topic. Whereas one study linked higher vitamin D levels with increased risk of flexural dermatitis (10), a Finnish population-based birth cohort found that maternal vitamin D intake during pregnancy was not associated with risk of atopic eczema when children were assessed at 5 years (11). Another birth cohort of 231 high-risk Australian infants observed that lower 25(OH)D levels in cord blood were more common among infants that developed eczema (12). Serum 25(OH)D levels were, however, not associated with allergen sensitization, IgE-mediated food allergy, and eczema severity as measured by SCORing Atopic Dermatitis (SCORAD).

A murine study showed that vitamin D receptor activation improved allergen-triggered eczema (13). However, clinical trials of vitamin D yielded conflicting results. Hata et al. (14) found among 14 eczema patients that daily administration of 4000 IU vitamin D increased expression of the antimicrobial peptide cathelicidin in lesional biopsies. Small case series suggested that vitamin D supplementation improved AD severity in children (15) and adults (16). However, the therapeutic efficacy of vitamin D on AD remains poorly defined in view of conflicting results reported in several randomized, double-blind, and placebo-controlled studies (17–19). In the present study, we measured serum 25(OH)D level in a case–control cohort of Chinese children to investigate the relationship between vitamin D and childhood AD.

Patients and methods

Study population

This case–control study consisted of 826 unrelated Hong Kong (22°N) Chinese children (498 AD cases and 328 non-allergic controls) (20). Patients with AD were younger than 18 years old, and their AD was diagnosed according to the criteria by Hanifin and Rajka (21). AD patients were recruited from both pediatric clinics of our university-affiliated teaching hospital ($n = 403$) and secondary schools that participated in a local epidemiological study for obesity and diabetes ($n = 95$) (20, 22). All controls did not report any AD, asthma and allergic rhinitis using the validated Chinese version of International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire (23), who were participants of our epidemiological study described above (20, 22). Short-term AD severity within preceding three days was determined by pediatric dermatologist using objective SCORAD (24), with the cutoff values for moderate and severe disease being 15 and 40, respectively. Long-term AD severity in the preceding 12 months was reported by caregivers using the self-administered Nottingham Eczema Severity Score (NESS) (25). Scores of 8 and 12 were the cutoffs for moderate and severe AD, respectively. These severity scores were only recorded in the subgroup of patients from our pediatric dermatology and allergy clinics. All subjects were free from any respiratory infection for 4 weeks before study. Subjects and their parents gave informed written consent, and Joint Chinese University

of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study.

Serum 25-hydroxyvitamin D measurement

The concentration of 25(OH)D in serum samples was measured using enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK). The intra- and interassay coefficients of variation were 3.3% and 6.5%. Serum 25(OH)D levels were shown by regression standardized residual plots to follow normal data distribution, which were categorized into deficient (<25 nm), insufficient (25–49.9 nm), and sufficient (≥ 50 nm) groups according to Institute of Medicine (IOM) reference (26).

Atopy markers

Plasma total immunoglobulin E (IgE) concentration was measured by microparticle immunoassay (IMx analyzer; Abbott Laboratories, Abbott Park, IL, USA), which was logarithm-transformed before analysis ($\log$ IgE). Increased IgE levels were determined based on local age-specific references. Circulating eosinophils were enumerated using a Coulter STKS counter (Beckman-Coulter, Miami, FL, USA) and were expressed in terms of percentage of total leukocytes (eos%). Eos% $\geq 5\%$ was considered to be increased (27). Children's atopy status was determined by positivity to at least one locally important allergen among house dust mites, cat, and cockroaches by skin prick test (SPT; wheal ≥ 3 mm larger than negative control) or specific IgE testing by radioallergosorbent test (≥ 0.35 kIU/l) (28, 29).

Statistical analysis

Data were expressed in number and percentage or mean and standard deviation (s.d.) as appropriate. Differences between cases and controls were analyzed by Student *t* test for numerical variables or χ^2 or Fisher's exact test for categorical data. Comparisons between groups were made using analysis of variance (ANOVA) in conjunction with Tukey's *post hoc* test. Age, sex, month of assessment, and batch of immunoassay measurements were included as covariates in the regression analysis for serum vitamin D level, and age and sex were adjusted for total IgE analysis. All comparisons were made two-tailed using statistical package R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria), with the level of significance set at 0.05.

Results

Table 1 describes subjects' clinical characteristics. The mean (s.d.) age of AD patients and controls was 10.5 (3.9) and 12.3 (4.1) years, respectively. Both total IgE level and eos% were higher in cases than controls. On the other hand, serum 25(OH)D levels were higher in controls than patients (mean [s.d.]: 34.2 [14.5] vs. 28.9 [15.3] nm; $p < 0.001$). The proportion of subjects with serum 25(OH)D levels <50 nm was similar between cases and controls (88.8% vs. 87.8%; $p = 0.760$), but more AD

Table 1 Clinical characteristics in our atopic dermatitis (AD) patients and controls

	AD (n = 498)	Control (n = 328)	p-value
Age, years	10.5 ± 3.9	12.3 ± 4.1	<0.001
Male	287 (57.6)	187 (57.0)	0.917
25(OH)D, nM	28.9 ± 15.3	34.2 ± 14.5	<0.001
LogIgE	2.90 ± 0.74	1.95 ± 0.68	<0.001
Increased plasma total IgE	416 (83.7)	117 (35.8)	<0.001
Atopy*	433 (93.1)	156 (47.6)	<0.001
Peripheral blood eosinophils†, %	8 (5–11)	ND	–

25(OH)D, 25-hydroxyvitamin D; LogIgE, logarithm-transformed plasma total IgE level; ND, not done.

Results presented in mean ± s.d. or number (percentage) unless stated otherwise.

*Defined as positive results by either skin prick tests or allergen-specific IgE in peripheral blood.

†Expressed as median (interquartile range).

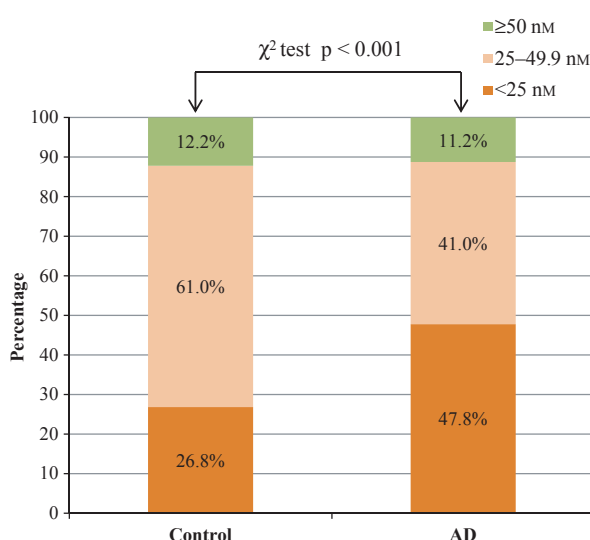
patients had serum 25(OH)D levels <25 nM than controls (47.8% vs. 26.6%; Fig. 1).

Data for SCORAD and NESS were available for 306 and 290 AD cases, respectively. Patients with and without severity data were similar for age, sex, and proportions with increased total IgE and eos% ($p > 0.1$ for all). One hundred and four, 129 and 73 patients had mild, moderate and severe AD by SCORAD, and 71, 103 and 116 patients by NESS, respectively. Serum 25(OH)D levels showed inverse associations with short-term and long-term AD severity (Fig. 2). Patients with vitamin D deficiency had higher LogIgE (3.08 ± 0.76) than those in the insufficient or sufficient groups (Fig. 3). Besides, the percentage of control subjects with increased total IgE was inversely related to serum 25(OH)D groups ($p = 0.040$; Table 2). Such association was not found among patients. Atopy was in general not associated with vitamin D status. Nonetheless, differences in atopy percentage between AD and controls observed at lower serum 25(OH)D levels were not detected among subjects whose levels exceeded 60 nM (Fig. 4).

Discussion

This case-control study provides the first evidence for the association between vitamin D and AD in the Chinese population. High prevalence of vitamin D deficiency was found in Hong Kong children and adolescents. Specifically, serum 25(OH)D levels were lower in AD than controls, which also showed inverse correlation with both SCORAD and NESS for disease severity. Furthermore, vitamin D-deficient patients had higher logIgE than those with insufficient and sufficient serum 25(OH)D levels.

Despite the well-known effect on bone health, vitamin D pathway emerged as a new regulatory mechanism for immune responses (3). There are two sources of vitamin D, with the

**Figure 1** Distribution of different status of serum 25(OH)D levels in AD patients and controls (p-value obtained by χ^2 test).**Table 2** Association between atopy markers and groups of serum 25 (OH)D levels

Vitamin D status	AD	Control
Atopy		
Deficient (<25 nM)	93.6%	46.6%
Insufficient (25–50 nM)	93.2%	46.0%
Sufficient (>50 nM)	90.7%	57.5%
p-value*	0.755	0.404
Increased plasma total IgE		
Deficient (<25 nM)	86.5%	43.2%
Insufficient (25–50 nM)	81.4%	35.7%
Sufficient (>50 nM)	80.4%	20.0%
p-value*	0.269	0.040
Increased eos%		
Deficient (<25 nM)	78.6%	ND
Insufficient (25–50 nM)	76.4%	ND
Sufficient (>50 nM)	75.6%	ND
p-value*	0.857	–

AD, atopic dermatitis; eos%, percentage of eosinophils among leukocytes in peripheral blood; ND, not done.

*Analyzed by χ^2 tests separately for control and AD groups.

major one being the conversion of 7-dehydrocholesterol to pre-vitamin D3 by ultraviolet-B from sunlight exposure. Intake of vitamin D3 from foods such as oily fish and cod liver oil is the other source. Inactive vitamin D is then metabolized to the major circulating form 25(OH)D, with a long half-life of more than two weeks, by CYP2R1 in microsomes and CYP27A1 in liver that encode 25-hydroxylase. The activated form of vitamin D ($1\alpha,25(\text{OH})_2\text{D}$ or calcitriol) is made following further hydroxylation step at C-1 position, which is transported via circulation to the nuclei of target cells by the group-specific components vitamin D-binding protein. The binding of $1\alpha,25(\text{OH})_2\text{D}$ to vitamin D receptor (VDR) results in hetero-

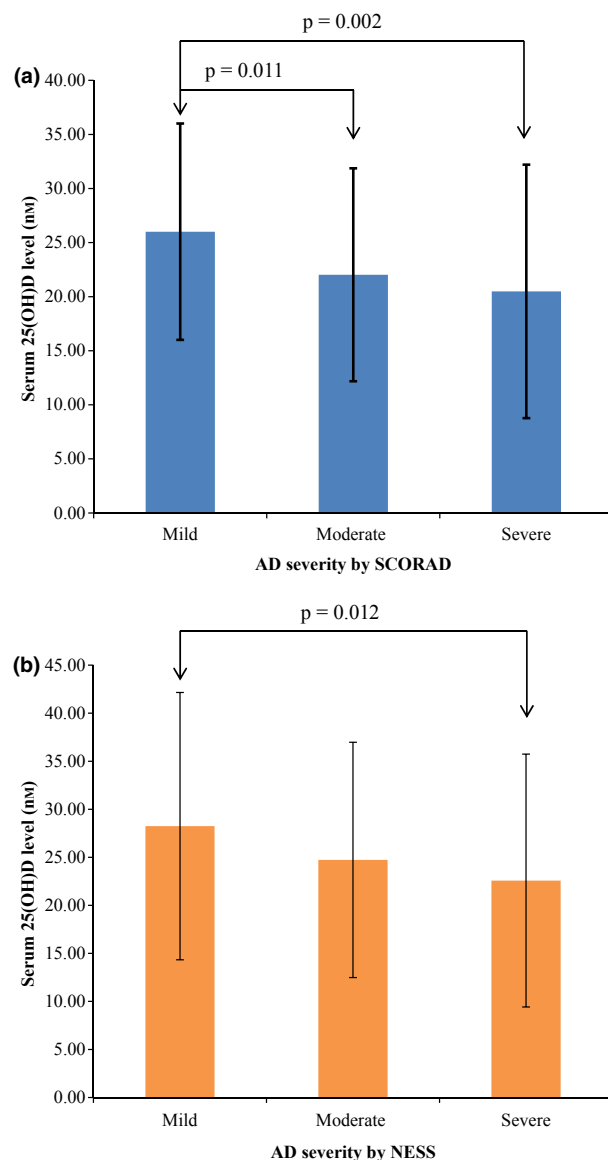


Figure 2 Relationship between serum 25(OH)D levels and (a) short-term AD severity by SCORing Atopic Dermatitis (SCORAD) and (b) long-term AD severity by NESS. The respective p-values obtained by multivariate regression were 0.0004 and 0.004 following adjustment for age, sex, month of assessment, and batch of immunoassay measurements as covariates.

dimerization with retinoid X receptors and regulation of the transcription of vitamin D responsive genes (30).

Our study found vitamin D deficiency to be strongly associated with childhood AD, and serum 25(OH)D levels inversely correlated with short-term and long-term disease severity. These results were consistent with those reported in a recent Italian study (8). A number of clinical trials supported that targeting vitamin D metabolism and signaling might be an effective treatment for AD. Sidbury et al. (15) recruited 11 children with mostly mild AD to receive ergocalciferol 1000 IU

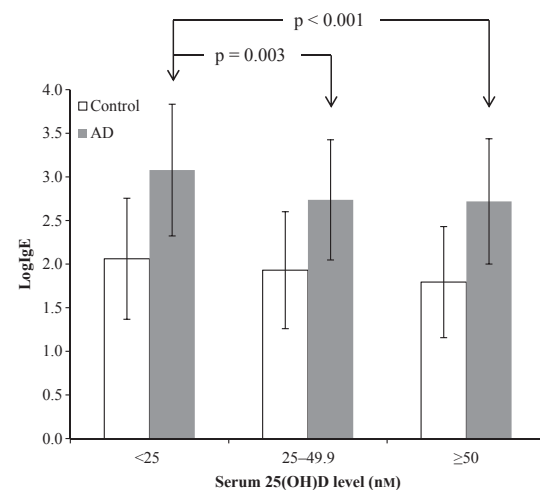


Figure 3 Differences in logarithm-transformed total IgE level among subjects with different groups according to serum 25(OH)D levels. p-values for trend in AD patients and controls were <0.001 and 0.092, respectively. Between-group differences were analyzed by Tukey's *post hoc* test.

or an identical-looking placebo once daily for 1 month. Vitamin D-treated patients had significant improvement in investigator's global assessment but not disease severity. Another short-term clinical trial found 1600 IU vitamin D daily to reduce disease severity in adults with AD (17). In a case series of 20 Caucasian adults who had low 25(OH)D levels and history of skin infections and winter AD exacerbations, Samchocki et al. (16) reported that a 3-month supplementation with 2000 IU/day cholecalciferol improved their disease severity. Such benefit might be explained by the observations that $1\alpha,25(\text{OH})_2\text{D}$ increased *in vitro* cathelicidin expression and antimicrobial activity in keratinocytes (31), which can help to restore an effective epidermal barrier. The frequency of bacterial skin infections was also higher in AD patients with lower serum 25(OH)D levels (16). Daily administration of 4000 IU vitamin D enhanced cathelicidin expressions in a case series of 14 eczema patients (14). The same group subsequently randomized 30 subjects with AD, 30 non-atopic subjects, and 16 subjects with psoriasis to receive 4000 IU of cholecalciferol or placebo for 21 days (18). In contrary to their earlier findings, there was no change in skin cathelicidin or AD severity among vitamin D-treated subjects despite an increase in serum 25(OH)D levels. On the other hand, another randomized, double-blind, placebo-controlled clinical trial showed that AD patients receiving daily dose of 1600 IU cholecalciferol for 60 days had significant improvement in SCORAD and Three Item Severity score (19). Larger randomized controlled trials of longer duration are thus needed to delineate the benefits of vitamin D treatment for AD.

There is no universal agreement about the cutoff value of serum 25(OH)D level that defines vitamin D deficiency. Our study followed IOM's recommendation to define vitamin D sufficiency at the 50 nm cutoff for serum 25(OH)D, whereas others suggested a cutoff of 75 nm (32). Ethnicity was a major factor that determines vitamin D level (18, 33). Several groups

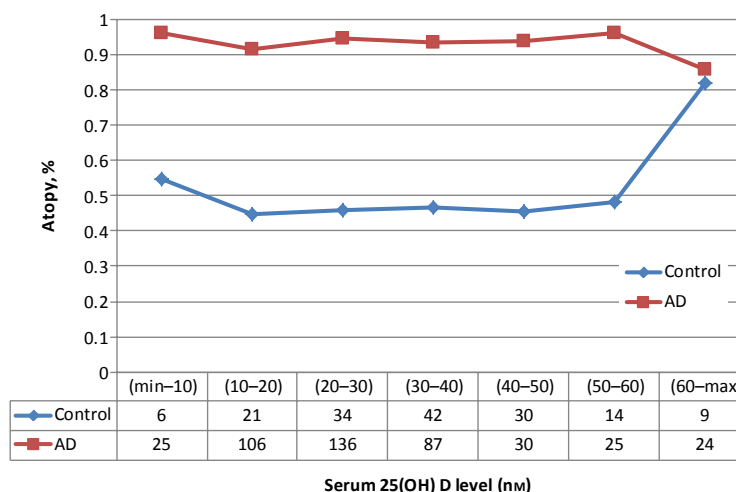


Figure 4 Relationship between percentage of atopy in AD patients and controls and serum 25(OH)D levels. At vitamin D level >60 nm, atopy percentage decreased in cases and increased in controls so that the between-group difference was insignificant.

reported high prevalence of vitamin D deficiency in Chinese. At 75 nm cutoff, a study in Hangzhou (latitude: 30°N) found vitamin D deficiency in nearly 90% of schoolchildren (34). Another study of 332 healthy adolescent girls in Beijing (40°N) also reported a prevalence of 90% for vitamin D deficiency at 50 nm cutoff. Based on the threshold 25 nm, this study found 47.8% and 26.6% for vitamin D deficiency among AD patients and non-allergic controls, respectively (Fig. 1). Our findings supported that vitamin D deficiency was also prevalent in Hong Kong Chinese children.

It is worth noting that the above cutoff values for vitamin D deficiency are based primarily on researches on bone health, whereas the optimal threshold for vitamin D level that reflects normal immunity remains unknown. Accordingly, this study compared serum 25(OH)D levels between cases and non-allergic controls. Nearly half of patients had serum 25(OH)D level <25 nm, which was 80% higher than controls. Besides, serum 25(OH)D level correlated inversely with both short-term and long-term disease severity in our patients. Total IgE was also higher among patients with low serum 25(OH)D levels, and the proportion of subjects with increased total IgE decreased with increasing 25(OH)D levels in our controls. These results supported an association between atopy and vitamin D deficiency (35).

The main drawback of this study relates to the unavailability of clinical factors such as subjects' physical activity, duration of outdoor and thus sunlight exposure, and detailed dietary intakes that regulate vitamin D homeostasis. Prospective cohorts are needed to accurately address the influences of these confounders on vitamin D deficiency. Another limitation is caused by the choice of laboratory assay for measuring 25(OH)D. The International Vitamin D External Quality Assessment Scheme compared 25(OH)D results obtained by different methods with the all-laboratory trimmed mean (ALTM). The immunoassay that we used in this study yielded results close to the target value, but which were lower than ALTM across the whole range. Bias was evident at 25(OH)D concentration >80 nm (36). To investigate this issue in this study, we randomly selected 10 samples in each of low, medium, and high ranges for serum 25(OH)D levels. Such samples were re-analyzed using liquid chromatography mass spectrophotometry, which yielded higher 25(OH)D levels

than by our immunoassay (means in nm: 48.1 vs. 41.5). Thus, this study overestimated to some extent the prevalence of vitamin D deficiency in Chinese children. Nonetheless, this methodological issue would not affect our results on case-control association between AD and vitamin D deficiency and relationship between serum 25(OH)D levels and AD severity. In addition, our AD patients were recruited from both pediatric clinics and local community, whereas the controls came only from the same community study. This raised the concern regarding the representativeness of our controls. From Table 1, the phenotypes of total IgE and atopy from allergen sensitization in both cases and controls were similar to what we reported in local hospital-based and community studies (20, 22, 37-39). These groups were matched for sex, but our controls were older than patients. The latter was expected given that our controls came from secondary schools. As atopic phenotypes are evolving in young children, secondary schools were selected as the reference population for our controls so that we were more confident that they were free from any ever history of allergic disease. Thus, we believe that our controls were representative of the general childhood population in Hong Kong.

In summary, vitamin D deficiency was associated with AD diagnosis and high total IgE levels in Hong Kong Chinese children. Vitamin D levels showed inverse correlation with both long- and short-term AD severity. Randomized placebo-controlled clinical trials are necessary to address the benefits of vitamin D treatment on AD outcomes.

Acknowledgments

We thanked Chung S. Ho and Michael H. M. Chan for performing LCMS to validate 25(OH)D levels. This study is funded by General Research Funds (469908 and 470909) of Hong Kong Research Grants Council, Research Committee Group Research Scheme (3110087), and Direct Grant for Research (2011.1.058) of Chinese University of Hong Kong.

Conflict of interest

The authors state no conflict of interest.

References

- Rosen CJ, Adams JS, Bikle DD, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev* 2012; **33**: 456–92.
- White JH, Vitamin D. signaling, infectious diseases, and regulation of innate immunity. *Infection Immun* 2008; **76**: 3837–43.
- Miller J, Gallo RL. Vitamin D and innate immunity. *Dermatol Ther* 2010; **23**: 13–22.
- Reinholz M, Ruzicka T, Schaubert J. Vitamin D and its role in allergic disease. *Clin Exp Allergy* 2012; **42**: 817–26.
- 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.
- Bieber T. Atopic dermatitis. *Ann Dermatol* 2010; **22**: 125–37.
- Wong GW, Hui DS, Chan HH, et al. Prevalence of respiratory and atopic disorders in Chinese schoolchildren. *Clin Exp Allergy* 2001; **31**: 1225–31.
- Peroni DG, Piacentini GL, Cametti E, Chinellato I, Boner AL. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol* 2011; **164**: 1078–82.
- Allen KJ, Koplin JJ, Ponsonby AL, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013; **131**: 1109–16.
- Tolppanen A-M, Sayers A, Granel R, Fraser WD, Henderson J, Lawlor DA. Prospective association of 25-hydroxyvitamin D3 and D2 with childhood lung function, asthma, wheezing, and flexural dermatitis. *Epidemiology* 2013; **24**: 310–9.
- Erkkola M, Kaila M, Nwaru BI, et al. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. *Clin Exp Allergy* 2009; **39**: 875–82.
- Jones AP, Palmer D, Zhang G, Prescott SL. Cord blood 25-hydroxyvitamin D3 and allergic disease during infancy. *Pediatrics* 2012; **130**: e1128–35.
- Hartmann B, Riedel R, Jörss K, et al. Vitamin D receptor activation improves allergen-triggered eczema in mice. *J Invest Dermatol* 2012; **132**: 330–6.
- Hata TR, Kotol P, Jackson M, et al. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J Allergy Clin Immunol* 2008; **122**: 829–31.
- Sidbury R, Sullivan AF, Thadhani RI, 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.
- Samochocki Z, Bogaczewicz J, Jeziorowska R, et al. Vitamin D effects in atopic dermatitis. *J Am Acad Dermatol* 2013; **69**: 238–44.
- Javanbakht MH, Keshavarz SA, Djalali M, et al. Randomized controlled trial using vitamins E and D supplementation in atopic dermatitis. *J Dermatolog Treat* 2011; **22**: 144–50.
- Hata TR, Audish D, Kotol P, et al. A randomized controlled double-blind investigation of the effects of vitamin D dietary supplementation in subjects with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2013; May 3. doi: 10.1111/jdv.12176 [Epub ahead of print].
- Amestajani M, Salehi BS, Vasigh M, et al. Vitamin D supplementation in the treatment of atopic dermatitis: a clinical trial study. *J Drugs Dermatol* 2012; **11**: 327–30.
- Wang SS, Hon KL, Sy HY, et al. Interactions between genetic variants of *FLG* and chromosome 11q13 locus determine susceptibility for eczema phenotypes. *J Invest Dermatol* 2012; **132**: 1930–2.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **92**: 44–7.
- Leung TF, Sy HY, Ng MC, et al. Asthma and atopy are associated with chromosome 17q21 markers in Chinese children. *Allergy* 2009; **64**: 621–8.
- Wong GW, Leung TF, Ko FW, et al. Declining asthma prevalence in Hong Kong Chinese schoolchildren. *Clin Exp Allergy* 2004; **34**: 1550–5.
- Stalder JF, Taieb A. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; **186**: 23–31.
- Hon KL, Ma KC, Wong E, Leung TF, Wong Y, Fok TF. Validation of a self-administered questionnaire in Chinese in the assessment of eczema severity. *Pediatr Dermatol* 2003; **20**: 465–9.
- Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. *Public Health Nutr* 2011; **14**: 938–9.
- Leung TF, Tang NL, Li CY, Lam CW, Wong GW, Fok TF. Association between *TARC* C-431T and atopy and asthma in children. *J Allergy Clin Immunol* 2004; **114**: 199–202.
- Hon KL, Leung TF, Ching G, et al. Patterns of food and aeroallergen sensitization in childhood eczema. *Acta Paediatr* 2008; **97**: 1734–7.
- Chan IH, Tang NL, Leung TF, et al. Study of gene-gene interactions for endophenotypic quantitative traits in Chinese asthmatic children. *Allergy* 2008; **63**: 1031–9.
- Henry HL. Regulation of vitamin D metabolism. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 531–41.
- Wang TT, Nestel F, Bourdeau V, et al. 1, 25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004; **173**: 2909–12.
- Holick MF. Vitamin D. deficiency. *New Engl J Med* 2007; **357**: 266–81.
- Dong Y, Pollock N, Stallmann-Jorgensen IS, et al. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. *Pediatrics* 2010; **125**: 1104–11.
- Zhu Z, Zhan J, Shao J, et al. High prevalence of vitamin D deficiency among children aged 1 month to 16 years in Hangzhou, China. *BMC Public Health* 2012; **12**: 126.
- Litonjua AA. Vitamin D. deficiency as a risk factor for childhood allergic disease and asthma. *Curr Opin Allergy Clin Immunol* 2012; **12**: 179–85.
- Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. *Clin Chem* 2004; **50**: 2195–7.
- Leung TF, Kong AP, Chan IH, et al. Association between obesity and atopy in Chinese schoolchildren. *Int Arch Allergy Immunol* 2009; **149**: 133–40.
- Leung TF, Li CY, Kong AP, et al. *PTGDR* is not a major candidate gene for asthma and atopy in Chinese children. *Pediatr Allergy Immunol* 2009; **20**: 556–62.
- Leung TF, Ching KW, Sy HY, et al. *CHIA* confers susceptibility to childhood eczema. *Br J Dermatol* 2010; **163**: 1360–2.