Original Article

Association of blood eosinophilia and vitamin D insufficiency in young infants with cow milk allergy

Jingwen Li MD, Xiaoli Mei MD, Xinyi Cai MD, Yujie Zhuo MD, Lanfang Zhang MD, Hongmei Guo MD, PhD, Hui Yang MD, Guang Yang MD, PhD

Children's Hospital of Nanjing Medical University, Nanjing, China

Background and Objectives: Cow milk allergy is the most common food allergic disease in young infants and vitamin D has a critical role in regulating intestinal inflammation. **Methods and Study Design:** To determine roles of vitamin D in cow milk allergy, fifty-six young infants with cow milk allergy were enrolled. The serum 25-hydroxyvitamin D (25OHD), total and specific IgE, circulating regulatory T lymphocytes, and blood eosinophil counts were determined. **Results:** The serum 25OHD in cow milk allergy and age-matched infants were similar (68.3±38.9 nmol/L versus 72.9±33.1 nmol/L, $p>0.05$), 71% Cow milk allergy infants (40/56) had serum 25OHD lower than 75 nmol/L compared to 66% (37/56) in the controls. The cow milk allergy infants with 25OHD lower than 75 nmol/L had persistent blood eosinophilia and delayed resolution of symptoms after cow milk elimination compared to those with 25OHD above 75 nmol/L (odd ratio 3.7, 95% CI 1.1-12.6, $p<0.05$). The serum 25OHD inversely correlated with blood eosinophil counts after cow milk elimination ($r=-0.37$, $p<0.01$). Cow milk allergy infants with 25OHD lower than 50 nmol/L (vitamin D deficiency, n = 22) were in general at younger age (1.6±0.6 months) compared to infants with insufficient (50-75 nmol/L) or normal (≥75 nmol/L) group (4.3±1.2 and 4.6±0.9 months, respectively, $p<0.001$). **Conclusions:** Low serum vitamin D associates with persistent blood eosinophilia and symptoms in young cow milk allergy infants.

Key Words: cow milk allergy, vitamin D, eosinophilia, young infants, intestinal inflammation

INTRODUCTION

Cow milk allergy (CMA) is very common in infants, especially those under 6 months. The incidence rate of CMA in children of one year or younger was estimated at 2% to 6%.[1] The symptoms vary but include diarrhea and/or bloody stool due to intestinal inflammation caused by cow milk protein (CMP), usually characterized by eosinophils-dominant intestinal mucosal inflammation with or without mildly to moderately increased blood eosinophilia.[2,3] CMP can cause severe allergic inflammation in the intestinal mucosa, leading to mucosal damage and increased intestinal permeability and, subsequently, iron deficiency or anemia, malnutrition or even growth retardation.[4,5] CMA in early stage of infancy has been also linked to increased risks in related gastroenteropathies and in allergic reactions in later life,[3] due to increased leakage of gut pathogens and the induction of antigen specific T cells and IgE memory caused by CMP-mediated persistent intestinal inflammation.[6-8] Therefore, there is indeed an urgent need to identify risk factors associated with CMA in early infancy.

The risk factors associated with CMA in early infancy are less defined. In adults, vitamin D insufficiency, unhealthful dietary fat, the timing of exposure to foods, as well as genetics have been implicated as potentially risk factors to food allergens.[9] Among them, vitamin D is a potential modifiable environmental and social factor which has been linked to intestinal inflammation.[10] Traditionally recognized as a central regulator to maintain mineral and bone homeostasis, vitamin D has recently been revealed to be involved in a number of aspects of intestinal inflammation and maintaining intestinal epithelial homeostasis.[11-13] Vitamin D deficiency can attenuate innate immune pathway of antimicrobial and anti-inflammatory response in the gut.[14] Vitamin D receptor signaling also suppresses NF-κB pathway of gut mucosal inflammation,[15] indicating that vitamin D status may affect allergic response in the gut. Low vitamin D level predisposes to more-severe and persistent intestinal inflammation.[16] It has been reported that vitamin D deficiency associates with allergic disorders in young children.[17,18] Low maternal and cord blood vitamin D levels have been linked to childhood allergy in general and has been found in CMA infants aged 2 years or younger.[19-21] Since infants with CMA are usually characterized by eosinophils-dominant intestinal mucosal inflammation with or without mildly to moderately increased blood eosinophilia,[2,3] we investigated serum vitamin D status and its impacts on serological or allergic parameters in CMA

Corresponding Author: Dr Guang Yang, Children's Hospital of Nanjing Medical University, 72 Guangzhou Road, Nanjing, Jiangsu 210008, China.
Tel: (+86) 25-83117361; Fax: (+86) 25-83117361
Email: gyangnj@qq.com
Manuscript received 19 February 2019. Initial review completed 02 June 2019. Revision accepted 30 June 2019.
doi: 10.6133/apjcn.201909_28(3).0014

infants.

METHODS

Study population and ethic statement

Infants under 6 months old who were brought to the Gastroenterology Clinic of Nanjing Children's Hospital (Nanjing, Jiangsu) from May 2015 to April 2018 were diagnosed with CMA based on clinical symptoms, negative search for other causes of the gastrointestinal symptoms and response to intact CMP elimination, in conformity with the national guideline adopted from the ones of World Allergy Organization (WAO) and other countries,[22,23] and the recently updated international guideline.[24] The infants with suspected CMA were given a casein hydrolysate and amino acids formula or CMP was eliminated from the mother's diet. After complete disappearance of clinical symptoms, CMP was reintroduced into the infants' or their mother's diet. Fifty-six infants with positive response to cow milk reintroduction were enrolled in this study following the procedure as was depicted in Figure 1. Infants with the following medical conditions were excluded from the study: previous history of medical/surgical problems, premature, current bacterial enterocolitis, severe dehydration or the need for hospitalization, or they were on a course of any drug treatment such as antibiotics, corticosteroids, or anti-reflux medication. Age-matched healthy infants whose blood samples were collected around the same time frame at the Child Health Clinic of the Hospital were included as controls.

The study was approved by the Clinical Research Ethics Committee of Nanjing Children's Hospital (protocol #201412001-1). Informed consent was obtained from a parent or a legal guardian of all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

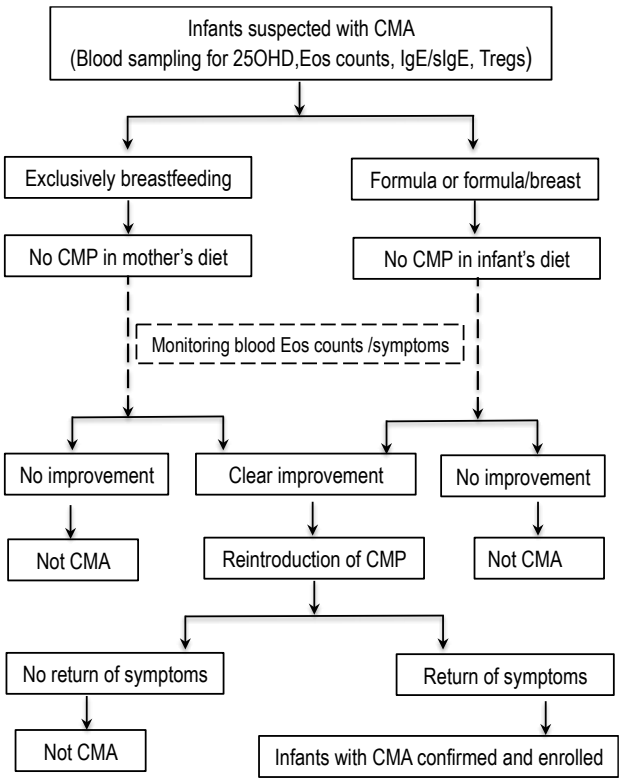

Figure 1. Diagram on the criterion used for patient with CMA recruitment. CMP, cow milk protein; Eos, eosinophils; sIgE, specific IgE; Tregs, regulatory T lymphocytes.

25-hydroxyvitamin D quantification by enzyme-linked immunoassay

Serum 25-hydroxyvitamin D (25OHD) reflects both vitamin D metabolic status and the contributions from all sources of vitamin D.[25] Blood samples were collected and stored frozen at -80°C until further analysis for serum 25OHD using an EIA kit (IDS, Boldon, UK) following the manufacturer's instructions.

Analysis of circulating populations of CD4+ lymphocytes and regulatory T cells

Proportion of $CD4^+$ lymphocytes and regulatory T lymphocytes (Tregs) in blood were measured as described.[26] Briefly, whole blood (100 μL) was incubated with 20 μL of a staining cocktail (Cat. No. 560249, BD Biosciences, CA, USA) that contains FITC anti-human CD4 antibody, PE-Cy7 anti-human CD25 antibody and Alexa Fluor-647 anti-human CD127 antibody for 20 minutes at room temperature. Erythrocytes were removed by adding 2 mL of red blood cell lysing buffer (Cat. No. 349202, BD Biosciences). The samples were analyzed on BD FACS ARIA II with FACS express software.

Measurement of serum allergic parameters

Total and specific immunoglobulin E against food allergens (CMP, egg, soybean, cod, peanut, and wheat flour) were measured using ImmunoCAP (Pharmacia Diagnostics, Freiburg, Germany). sIgE values ≥0.35 kU/L were used to define allergic sensitization.

A complete blood cell counts with differential for evaluation of peripheral eosinophilia before and after CMP removal from infants' diet was analyzed using a Coulter STKS counter (Beckman Coulter, Miami, Florida), and peripheral absolute eosinophil count was expressed as absolute number ($\times 10^9$ cells/L). Blood eosinophilia is defined when eosinophil count is more than 0.5×10^9 cells/L.[27]

Statistical analysis

Descriptive data are presented appropriately as mean ± SD, number and frequency (%). Unpaired or paired sample t-test (for two groups) or one-way ANOVA (for multiple groups) and Fisher's exact test (Chi-square tests) were used to analyze the differences of clinical and immunological variables based on serum vitamin D status. Bivariate relationships between vitamin D levels and immunological variables were assessed using Spearman's correlation test. Additionally, multivariable logistic regression was performed to assess vitamin D status or other factors independently related to the odds ratio (OR) of complete resolution of symptoms after 1-week cow milk elimination. The variables were entered into the model to adjust for potential confounding variables including vitamin D levels, age, and immunological parameters. All statistical analyses were performed using SPSS software

(SPSS Inc., Chicago, Illinois), and *p* values of less than 0.05 were considered to be statistically significant.

RESULTS

Fifty-six infants who were diagnosed with CMA were enrolled in this study and 56 age-matched healthy infants were included as healthy controls. A retrospective investigation showed that all infants received CMP by cow milk formula or breast milk from mothers who themselves consumed cow's milk protein in the maternal diet. The clinical symptoms of those infants were mainly diarrhea and or bloody stool.

We found that serum 25OHD in infants with CMA and the control varied widely. However, there was no significant difference in mean serum 25OHD between the two groups (p=0.494) (Table 1, Figure 2A). To correlate serum 25OHD to the disease, we grouped the participants based on their serum 25OHD using the recommended 75 nmol/L and 50 nmol/L as the cutoff points.[28] Seventy-one percent (40/56) of the CMA infants and 66% (37/56) of the controls had serum 25OHD lower than 75 nmol/L. A positive association between age and serum 25OHD (r=0.725, p<0.001, Figure 2B) was detected in CMA patients of early infancy. It is noteworthy that CMA infants with 25OHD lower than 50 nmol/L (vitamin D deficiency, n=22) were at younger age in general (1.6±0.6 versus 4.3±1.2 and 4.6±0.9 months in vitamin D insufficient and normal groups, respectively, p<0.001, Table 2). The age effect was less prominent between vitamin D insufficiency (25OHD at 50-75 nmol/L) and vitamin D normal groups (25OHD at 75 nmol/L or above, Table 2).

Many CMA infants in our study had mildly to moderately elevated eosinophil counts (AEC1, Table 2). One week after cow milk elimination, the eosinophil counts of infants with normal 25OHD group returned to normal levels (AEC1 versus AEC2, p=0.003, Table 2) whereas those with low 25OHD remained unchanged (AEC1 versus AEC2, p=0.665 and 0.348, respectively, for the deficient and insufficient group). A binary regression analysis showed that serum 25OHD inversely correlated with the peripheral eosinophil counts after 1-week cow milk elimination (r=-0.371, p=0.005, Figure 3). All infants showed improvement of symptoms upon cow milk-free diet and some had no symptoms at all after 1-week follow-up. However, statistical analysis showed that low 25OHD was associated with delayed resolution of symptoms after allergen elimination (odd ratio 3.7, 95% CI 1.1-12.6, p=0.036). The results indicated that vitamin D deficiency or insufficiency negatively impacted the disease process.

No infant in the study had detectable sIgE for CMP, along with their clinical history and symptoms, which suggested that all the studied infants had mild to moderate non-IgE-mediated CMA. However, early infancy is the key period for the development of adaptive immune response.[29] We also investigated the changes of IgE and Tregs and correlated those changes to serum 25OHD in those infants with CMA (Table 2). However, some CMA infants had "increased" total IgE level as was defined by Weisse and colleagues.[30] Infants with serum 25OHD lower than 50 nmol/L had significantly lower total IgE compared to those with insufficient or normal serum vitamin D (p<0.001 and p=0.003, respectively). Total IgE were higher in vitamin D insufficient group than that in vitamin D normal group, but the effect was not significant. No correlation between serum 25OHD to the population of circulatory Tregs.

DISCUSSION

Cow milk allergy is the most common food allergic disorder during infancy and the onset of the disorder usually begins at the age under 6 months and remains at the highest prevalence during the first year after birth.[31] It is also one of the most complex food allergies, being implicated in IgE-mediated food allergy as well as diverse manifestations of non-IgE-mediated food allergy.[24] This study focused on young infants under 6 months of age who had mild to moderate non-IgE-mediated CMA. The criteria used for patient recruitment was similar to the published guidelines from the United Kingdom, Australia, and many other countries.[22,23,32,33] We found that low serum vitamin D was associated with persistent blood eosinophilia and symptoms in young cow milk allergy infants.

Low vitamin D status in pregnant women or neonate has adverse health effects,[34] and is also a risk factor for childhood food allergy.[18] Silva and colleagues reported lower serum vitamin D in CMA infants aged 2 years or younger, especially those who were exclusively or predominantly breastfed.[21] We found a strong correlation of low serum vitamin D to persistent blood eosinophilia in CMA infants in early infancy. These and our study make vitamin D deficiency and insufficiency a possible risk factor for CMA.

Table 1. Characteristics of infants with CMA and age-matched controls

	CMA (n=56)	Control (n=56)
Age (month)	3.3±1.7 (0.7-5.8)	3.1±1.4 (0.4-5.9)
Male/Female (n)	30/26	29/27
Mean 25OHD level (nmol/L)	68.3±38.9	59.4±38.1
25OHD range (nmol/L, range)	14.4-150	13.4-163
25OHD <75 nmol/L (%)	71 (40/56)	66 (37/56)
AEC ($\times 10^9$/L)	0.89±0.45 (0.26-2.67)	0.26±0.12**
Breast milk/Mix/cow milk (n)	20/26/10	25/22/9
VitD supplementation (%)	73 (41/56)	85% (48/56)
Time of birth (AW/SS)	30/16	30/16
Weight at birth (kg)	3.44±0.39	3.45±0.38

AEC: peripheral absolute eosinophil counts; AW: autumn and winter; SS: spring and summer; VitD: vitamin D.
Data are shown in mean±SD, range, number or frequency appropriately.
Statistically differences are indicated in *(p<0.05) and **(p<0.01).

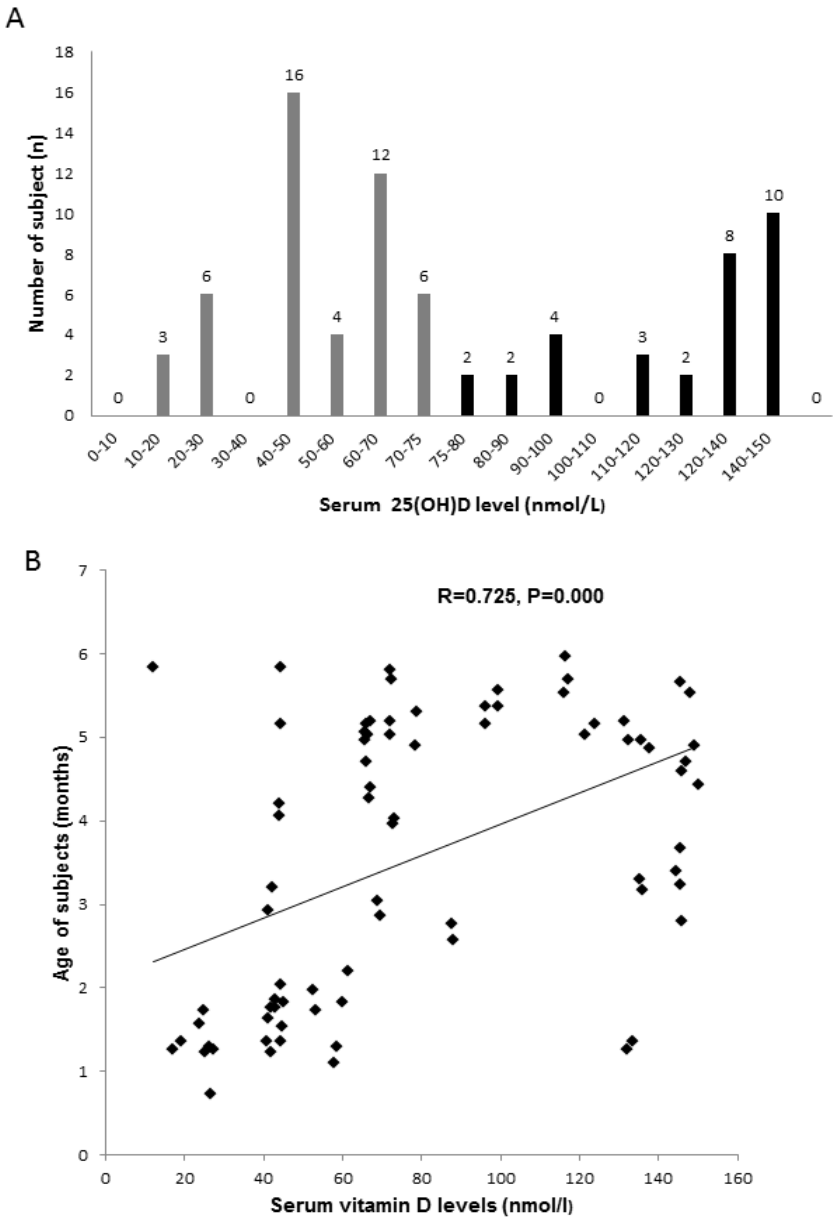

Figure 2. Distribution of serum vitamin D concentration ranges in infants with cow milk allergy and the association with age of infants. A. Distribution of serum vitamin D ranges. The number above each bar represents the total number of children having a vitamin D with each range. B. Correlation of serum vitamin D concentrations to age.

Table 2. Comparisons of infants with CMA based on serum 25OHD

	<50 nmol/L (deficient, n=22)	50-75 nmol/L (insufficient, n=18)	≥75 nmol/L (normal, n=16)
25OHD (nmol/L)	32.38±10.95**	67.03±5.99*	118.99±26.90
Age (month)	1.6±0.6**	4.3±1.2	4.6±0.9
Male/Female (n)	10/12	10/8	8/8
Duration (weeks)	2.0±0.8*	2.3±0.6	3.0±1.7
Breast/mix/cow milk (n)	11/9/2	5/7/6	5/9/2
Weight gain (kg/month)	1.09±0.45	1.10±0.67	0.83±0.23
VitD supplementation (n)	18	10	13
Tregs (%)	9.1±1.8	7.7±2.1	8.9±2.3
Total IgE (kU/L)	4.35±3.87**	12.88±8.66	10.37±5.02
AEC1 ($\times 10^9$ cells/L)	0.99±0.25	0.67±0.29	1.03±0.67
AEC2 ($\times 10^9$ cells/L)	0.91±0.62**	0.65±0.21*	0.39±0.15
No/yes symptoms (n)†	7/15	8/10	11/5

VitD: vitamin D. AEC1 and AEC2, peripheral absolute eosinophil counts before and after1-week cow milk elimination, respectively.
Data are shown in mean±SD, range, number or frequency appropriately.
Statistically differences are indicated in *($p<0.05$) and **($p<0.01$).
†no/yes symptoms, numbers of patients with symptoms disappeared/persisted after 1-week CMP elimination.

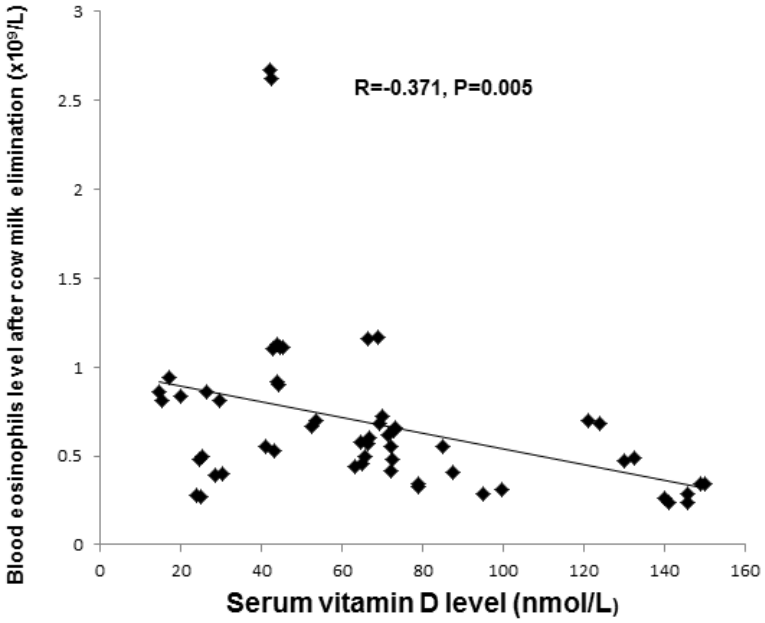

Figure 3. Correlation between serum 25OHD and blood eosinophil counts in infants with CMA. Binary regression analysis showed that serum 25OHD blood eosinophil and vitamin D was inversely correlated with blood eosinophil counts after cow milk elimination in infants with CMA.

It is known that neonatal vitamin D concentration relies on maternal vitamin D status and the ability of the placenta to transport.[35] Vitamin D insufficiency is common among pregnant women and inadequate vitamin D intake during pregnancy has been implicated in low maternal and cord blood vitamin D concentrations.[36,37] We noticed that none of the mothers whose child had low serum vitamin D had taken vitamin D supplementation during pregnancy or lactation prior to clinic visitation, which could contribute to low serum vitamin D in those infants. Sunlight exposure stimulates the synthesis of vitamin D in skin and has an important role in maintaining vitamin D homeostasis. Due to customary traditions in China, newborns tend to stay indoors. Lacking of sunlight exposure may contribute to the deficiency or insufficiency of vitamin D in these infants since almost all the younger infants in this study had been kept indoor for at least 30 days after birth. Increasing evidence shows that vitamin D supplementation is as an effective measure to prevent vitamin D deficiency or insufficiency in individuals in many sunny or sunshine restricted areas.[38-41] In our study, most CMA infants began to receive vitamin D supplementation (at 400 IU/day of vitamin D) 2 weeks after birth except that a few CMA infants did not receive vitamin D supplementation intermittently due to diarrhea. Although malabsorption of vitamin D due to ongoing CMA may be a contributing factor for low vitamin D levels, we were unable to determine the causes of vitamin D insufficiency in those younger infants. A further study to closely monitor both infant and maternal vitamin D status and the disease may strengthen the argument on the importance of maternal vitamin D intake on the pathogenesis of early infant CMA. It was recently reported that vitamin D supplementation during pregnancy had expected effects on maternal and infant serum 25OHD and calcium concentrations.[42] In a population with widespread prenatal vitamin D insufficiency, it will be interesting to know whether vitamin D supplementation during pregnancy may reduce CMA incidence to those who have a history to food allergy in general.

Vitamin D has multiple biological effects, and may directly or indirectly affects the blood eosinophils of CMA infants. Eosinophils are major innate immune cells being resident mainly in gut under steady-state, and play important roles in maintaining gut homeostasis and inflammation.[43,44] Being mature in and released from bone marrow, blood eosinophil level is often increased in allergic diseases and has been used as a marker for monitoring the process of allergic disorders in clinical settings.[45] Consistent with this, we found that infants with CMA had increased blood eosinophil numbers. During intestinal inflammation, increased eosinophilopoiesis occurs and eosinophils expand in bone marrow, usually resulting in increased gut-homing eosinophils being released into circulation.[44] Residential eosinophils in gut can be a direct target of CMP-induced cellular immune response.[46] Eosinophils migrate rapidly into inflammatory foci and sites of active tissue repair to exert their pro-inflammatory or protective action against invading exogenous pathogens from lumen of gastrointestinal tract.[47-49] Like other immune cells, eosinophils themselves express VDR (Vitamin D Receptor). Vitamin D can modulate eosinophils migration in a concentration-dependent manner and reduce the release of cytotoxic granules by eosinophils.[50,51] Vitamin D and its receptor mediated signaling has been involved in the proliferation of intestinal mucosal epithelial cells and maintaining intact intestinal barrier function,[13,52] which can affect the production of gut-derived chemokines and cytokines for recruiting eosinophils. Thus, vitamin D may directly or indirectly affect the resolution of blood eosinophilia and symptoms of CMA.

Vitamin D insufficiency in China is common among young children from rural areas as well as newborns from

big cities like Shanghai.[53,54] Vitamin D and derivatives originate naturally from exposure to sunlight; nevertheless, many factors can impair this process, necessitating periodic reliance on dietary sources to maintain adequate serum concentrations.[55] Because foods naturally rich in vitamin D are limited, in many countries, the general populations are largely dependent on fortified foods and dietary supplements to meet these needs.[56,57] Even in countries that do fortify, vitamin D intakes are low in some groups due to their unique dietary patterns or to the desire in avoiding sunlight exposure as a result of lifestyle preference in regions globally.[57] We found that low vitamin D status exists in CMA patients of early infancy. Coincidently, none of the mothers whose child had vitamin D insufficiency had vitamin D supplementation or fortified foods during their pregnancy. Vitamin D insufficiency appears to be a global problem in mothers and infants and vitamin D supplementation during pregnancy was associated with increased circulating 25OHD levels, birth weight and birth length.[58] Some groups have advocated for worldwide attention to monitor maternal and infant vitamin D status during pregnancy and lactation.[39,59] In addition to showing a correlation of vitamin D insufficiency to CMA in early infancy, this study also argues strongly for vitamin D normalization during pregnancy as well as in new mothers who are breastfeeding their babies.

This study had its limitations due to no data provided about the effects of low serum vitamin D on cytokines or chemokines that may lead to persistent blood eosinophilia in CMA infants. Monitoring or normalizing serum vitamin D status of these infants and understanding its relation with the response to reintroduction of CMP and CMP induced intestinal inflammation would provide stronger evidence for vitamin D involving in the progression of CMA.

In conclusion, this study revealed low serum vitamin D is associated with persistent blood eosinophilia and symptoms in young CMA infants. Further research should identify the mechanisms of low serum vitamin D on the migration and or activation of eosinophils as well as the effects of normalizing vitamin D status on the progression of CMA.

ACKNOWLEDGEMENTS

The authors acknowledge all the patients and their families in the Pediatric Gastroenterology Unit, Nanjing Children's Hospital, for their cooperation during the study period, and Dr Y Fan of Nanjing University Medical School for assistance with statistics.

AUTHOR DISCLOSURES

The authors declare no conflict of interest. The study was funded by a grant from Nanjing Municipal Department of Science and Technology (ZKX1804040 to GY). The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

REFERENCES

1. Mousan G, Kamat D. Cow's milk protein allergy. Clin Pediatr. 2016;55:1054-63. doi: 10.1177/0009922816664512.
2. Shah N, Foong RM, Borrelli O, Volonaki E, Dziubak R, Meyer R et al. Histological findings in infants with Gastrointestinal food allergy are associated with specific gastrointestinal symptoms; retrospective review from a tertiary centre. BMC Clin Pathol. 2015;15:12. doi: 10.1186/s12907-015-0012-6.
3. Meyer R, Fox AT, Chebar Lozinsky A, Michaelis LJ, Shah N. Non-IgE-mediated gastrointestinal allergies-Do they have a place in a new model of the Allergic March. Pediatr Allergy Immunol. 2019;30:149-58. doi: 10.1111/pai.13000.
4. Robbins KA, Wood RA, Keet CA. Milk allergy is associated with decreased growth in US children. J Allergy Clin Immunol. 2014;134:1466-8 e6. doi: 10.1016/j.jaci.2014.08.037.
5. Jhamnani RD, Levin S, Rasooly M, Stone KD, Milner JD, Nelson C et al. Impact of food allergy on the growth of children with moderate-severe atopic dermatitis. J Allergy Clin Immunol. 2018;141:1526-9 e4. doi: 10.1016/j.jaci.2017.11.056.
6. Vijayendra Chary A, Hemalatha R, Seshacharyulu M, Vasudeva Murali M, Jayaprakash D, Dinesh Kumar B. Vitamin D deficiency in pregnant women impairs regulatory T cell function. J Steroid Biochem Mol Biol. 2015;147:48-55. doi: 10.1016/j.jsbmb.2014.11.020.
7. Valenta R, Hochwallner H, Linhart B, Pahr S. Food allergies: the basics. Gastroenterology. 2015;148:1120-31 e4. doi: 10.1053/j.gastro.2015.02.006.
8. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol. 2014; 133:621-31. doi: 10.1016/j.jaci.2013.12.1088.
9. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141:41-58. doi: 10.1016/j.jaci.2017.11.003.
10. Vuillermin PJ, Ponsonby AL, Kemp AS, Allen KJ. Potential links between the emerging risk factors for food allergy and vitamin D status. Clin Exp Allergy. 2013;43:599-607. doi: 10.1111/cea.12048.
11. Ghaly S, Lawrance I. The role of vitamin D in gastrointestinal inflammation. Expert Rev Gastroenterol Hepatol. 2014; 8:909-23. doi: 10.1586/17474124.2014.925796.
12. Shang M, Sun J. Vitamin D/VDR, probiotics, and gastrointestinal diseases. Curr Med Chem. 2017;24:876-87. doi: 10.2174/0929867323666161202150008.
13. Kuhne H, Schutkowski A, Weinholz S, Cordes C, Schierhorn A, Schulz K et al. Vitamin D receptor regulates intestinal proteins involved in cell proliferation, migration and stress response. Lipids Health Dis. 2014;13:51. doi: 10.1186/1476-511X-13-51.
14. Wang TT, Dabbas B, Laperriere D, Bitton AJ, Soualhine H, Tavera-Mendoza LE et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. J Biol Chem. 2010;285:2227-31. doi: 10.1074/jbc.C109.071225.
15. Liu W, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. J Clin Invest. 2013; 123:3983-96. doi: 10.1172/JCI65842.
16. Assa A, Vong L, Pinnell LJ, Rautava J, Avitzur N, Johnson-Henry KC et al. Vitamin D deficiency predisposes to adherent-invasive Escherichia coli-induced barrier dysfunction and experimental colonic injury. Inflamm Bowel Dis. 2015; 21:297-306. doi: 10.1097/mib.0000000000000282.
17. Papadopoulou A, Bountouvi E, Papaevaggelou V, Priftis KN. Maternal vitamin D status and development of asthma

and allergy in early childhood. Mini Rev Med Chem. 2015; 15:900-12.
18. Querfeld U, Keil T, Beyer K, Stock P, Pilz S, Marz W et al. Vitamin D in early life: good or bad for food allergies? Allergy. 2013;68:1081-3. doi: 10.1111/all.12178.
19. Chiu CY, Huang SY, Peng YC, Tsai MH, Hua MC, Yao TC et al. Maternal vitamin D levels are inversely related to allergic sensitization and atopic diseases in early childhood. Pediatr Allergy Immunol. 2015;26:337-43. doi: 10.1111/pai. 12384.
20. Chiu CY, Yao TC, Chen SH, Tsai MH, Tu YL, Hua MC et al. Low cord blood vitamin D levels are associated with increased milk sensitization in early childhood. Pediatr Allergy Immunol. 2014;25:767-72. doi: 10.1111/pai.12304.
21. Silva CM, Silva SAD, Antunes MMC, Silva G, Sarinho ESC, Brandt KG. Do infants with cow's milk protein allergy have inadequate levels of vitamin D? J Pediatr. 2017;93:632-8. doi: 10.1016/j.jped.2017.01.006 .
22. Fiocchi A, Brozek J, Schunemann H, Bahna, SL, von Berg, A, Beyer K et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. World Allergy Organ J. 2010;3:57-161. doi: 10.1097/WOX.0b013e3181defeb9.
23. Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M et al. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. Arch Dis Child. 2007;92:902-8.
24. Venter C, Brown T, Meyer R, Walsh J, Shah N, Nowak-Wegrzyn A et al. Better recognition, diagnosis and management of non-IgE-mediated cow's milk allergy in infancy: iMAP-an international interpretation of the MAP (Milk Allergy in Primary Care) guideline. Clin Transl Allergy. 2017; 23;7:26, doi: 10.1186/s13601-017-0162-y.
25. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc. 2013;88:720-55. doi: 10. 1016/j.mayocp.2013.05.011.
26. Hrdy J, Kocourkova I, Prokesova L. Impaired function of regulatory T cells in cord blood of children of allergic mothers. Clin Exp Immunol. 2012;170:10-7. doi: 10.1111/j.1365-2249.2012.04630.x.
27. Klion AD. How I treat hypereosinophilic syndromes. Blood. 2015;126:1069-77. doi: 10.1182/blood-2014-11-551614.
28. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011; 96:1911-30. doi: 10.1210/jc.2011-0385.
29. Jones AP, D'Vaz N, Meldrum S, Palmer DJ, Zhang G, Prescott SL. 25-hydroxyvitamin D3 status is associated with developing adaptive and innate immune responses in the first 6 months of life. Clin Exp Allergy. 2015;45:220-31. doi: 10. 1111/cea.12449 .
30. Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. Allergy. 2013;68:220-8. doi: 10.1111/all.12081.
31. Høst A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol. 2002;89:33-7.
32. Allen KJ, Davidson GP, Day AS, Hill DJ, Kemp AS, Peake JE et al. Management of cow's milk protein allergy in infants and young children: an expert panel perspective. J Paediatr Child Health. 2009;45:481-6. doi: 10.1111/j.1440-1754. 2009.01546.x.
33. Caffarelli C1, Baldi F, Bendandi B, Calzone L, Marani M, Pasquinelli P et al. Cow's milk protein allergy in children: a practical guide. Ital J Pediatr. 2010;36:5. doi: 10.1186/1824-7288-36-5.
34. Bi WG, Nuyt AM, Weiler H, Leduc L, Santamaria C, Wei SQ. Association between vitamin D supplementation during pregnancy and offspring growth, morbidity, and mortality: a systematic review and meta-analysis. JAMA Pediatr. 2018; 172:635-45. doi: 10.1001/jamapediatrics.2018.0302.
35. Josefson JL, Feinglass J, Rademaker AW, Metzger BE, Zeiss DM, Price HE et al. Maternal obesity and vitamin D sufficiency are associated with cord blood vitamin D insufficiency. J Clin Endocrinol Metab. 2013;98:114-9. doi: 10. 1210/jc.2012-2882.
36. Harvey NC, Holroyd C, Ntani G, Javaid K, Cooper P, Moon R et al. Vitamin D supplementation in pregnancy: a systematic review. Health Technol Assess. 2014;18:1-190. doi: 10. 3310/hta18450.
37. Lundqvist A, Sandstrom H, Stenlund H, Johansson I, Hultdin J. Vitamin D status during pregnancy: a longitudinal study in Swedish women from early pregnancy to seven months postpartum. PLoS One. 2016;11:e0150385. doi: 10.1371/journal.pone.0150385.
38. Dawodu A, Zalla L, Woo JG, Herbers PM, Davidson BS, Heubi JE et al. Heightened attention to supplementation is needed to improve the vitamin D status of breastfeeding mothers and infants when sunshine exposure is restricted. Matern Child Nutr. 2014;10:383-97. doi: 10.1111/j.1740-8709.2012.00422.x.
39. Dawodu A, Davidson B, Woo JG, Peng YM, Ruiz-Palacios GM, de Lourdes Guerrero M, Morrow AL. Sun exposure and vitamin D supplementation in relation to vitamin D status of breastfeeding mothers and infants in the global exploration of human milk study. Nutrients. 2015;7:1081-93. doi: 10.3390/nu7021081.
40. Aghajafari F, Field CJ, Weinberg AR, Letourneau N, APrON Study Team. Both mother and infant require a vitamin D supplement to ensure that infants' vitamin D status meets current guidelines. Nutrients. 2018;10:E429. doi: 10. 3390/nu10040429.
41. Hlusko LJ, Carlson JP, Chaplin G, Elias SA, Hoffecker JF, Huffman M et al. Environmental selection during the last ice age on the mother-to-infant transmission of vitamin D and fatty acids through breast milk. Proc Natl Acad Sci U S A. 2018;115:E4426-32. doi: 10.1073/pnas.1711788115.
42. Roth DE, Morris SK, Zlotkin S, Gernand AD, Ahmed T, Shanta SS et al. Vitamin D supplementation in pregnancy and lactation and infant growth. N Engl J Med. 2018;379: 535-46. doi: 10.1056/NEJMoa1800927.
43. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and functions. Front Med (Lausanne). 2017;4: 101. doi: 10.3389/fmed.2017.00101 (2017).
44. Weller PF, Spencer LA. Functions of tissue-resident eosinophils. Nat Rev Immunol. 2017;17:746-60 doi: 10.1038/nri. 2017.95.
45. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. J Allergy Clin Immunol. 2015;135:822-4 e2. doi: 10. 1016/j.jaci.2014.09.034.
46. Jo J, Garssen J, Knippels L, Sandalova E. Role of cellular immunity in cow's milk allergy: pathogenesis, tolerance induction, and beyond. Mediators Inflamm. 2014;2014: 249784. doi: 10.1155/2014/249784.
47. Dent G, Loweth SC, Hasan AM, Leslie FM. Synergic production of neutrophil chemotactic activity by colonic epithelial cells and eosinophils. Immunobiology. 2014;219:793-7. doi: 10.1016/j.imbio.2014.06.008.
48. Chu VT, Beller A, Rausch S, Strandmark J, Zanker M, Arbach O et al. Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and

contribute to gut immune homeostasis. Immunity. 2014;40: 582-93. doi: 10.1016/j.immuni.2014.02.014 .
49. Travers J, Rothenberg ME. Eosinophils in mucosal immune responses. Mucosal Immunol. 2015;8:464-75. doi: 10.1038/mi.2015.2.
50. Hiraguchi Y, Tanida H, Sugimoto M, Hosoki K, Nagao M, Tokuda R et al. 1,25-Dihydroxyvitamin D3 upregulates functional C-x-C chemokine receptor type 4 expression in human eosinophils. Int Arch Allergy Immunol. 2012; 158(Suppl 1):51-7. doi: 10.1159/000337767.
51. Ethier C, Yu Y, Cameron L, Lacy P, Davoine F. Calcitriol reduces eosinophil necrosis which leads to the diminished release of cytotoxic granules. Int Arch Allergy Immunol. 2016;171:119-29. doi: 10.1159/000450951 (2016).
52. Li YC, Chen Y, Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. J Steroid Biochem Mol Biol. 2015;148:179-83. doi: 10.1016/j.jsbmb.2015.01.011.
53. Yu X, Wang W, Wei Z, Ouyang F, Huang L, Wang X et al. Vitamin D status and related factors in newborns in Shanghai, China. Nutrients. 2014;6:5600. doi: 10.3390/nu6125600.
54. Geng SS, Ma JQ, Liu SS, Zhang J, Sheng XY. Vitamin D insufficiency and its association with biochemical and anthropometric variables of young children in rural Southwestern China. Chin Med J (Engl). 2016;129:1273-9. doi: 10.4103/0366-6999.182834.
55. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. J Nutr. 2005;135:310-6. doi: 10.1093/jn/135.2.310.
56. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004;80(6 Suppl):1710S-6S. doi: 10.1093/ajcn/80.6.1710S.
57. Yang Z, Laillou A, Smith G, Schofield D, Moench-Pfanner R. A review of vitamin D fortification: implications for nutrition programming in Southeast Asia. Food Nutr Bull. 2013;34(Suppl 2):S81-9. doi: 10.1177/15648265130342S110.
58. Perez-Lopez FR, Pasupuleti V, Mezones-Holguin E, Benites-Zapata VA, Thota P, Deshpande A et al. Effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2015;103:1278-88 e4. doi: 10.1016/j.fertnstert.2015.02.019.
59. Mohamed SA, Al-Hendy A, Schulkin J, Power ML. Opinions and practice of US-based obstetrician-gynecologists regarding vitamin D screening and supplementation of pregnant women. J Pregnancy. 2016;2016:1454707. doi: 10.1155/2016/1454707.