HHS Public Access

Author manuscript

J Allergy Clin Immunol Pract. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

J Allergy Clin Immunol Pract. 2018 ; 6(1): 289–291.e2. doi:10.1016/j.jaip.2017.07.019.

Vitamin D insufficiency, plasma cytokines, and severe asthma exacerbations in school-aged children

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To the Editor

Severe asthma exacerbations lead to child distress, missed work days, and school absences. Such exacerbations account for approximately one third of the estimated $56 billion spent per year on asthma care (1).

In the U.S., Puerto Ricans share a disproportionate burden from asthma. We previously showed that vitamin D insufficiency (a plasma 25(OH)D <30 ng/ml) is associated with severe asthma exacerbations in Puerto Ricans, with stronger effects in children with non-atopic asthma than in those with atopic asthma (2).

On the basis of our findings and those of others, we hypothesized that vitamin D insufficiency leads to severe asthma exacerbations through mechanisms other than altered Th2 immune responses (3). To test this hypothesis, we examined vitamin D insufficiency, plasma cytokines, and severe asthma exacerbations in Puerto Rican children.

Please also see the Online Repository. 678 children aged 6–14 years were recruited in San Juan (PR) using a multistage probability sample design (2). Only those with data on plasma vitamin D and cytokine levels (n=578) are included. Study protocol included questionnaires and collection of blood samples. Written parental consent and assent from participating children were obtained, and the study was approved by Institutional Review Boards.

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Conflicts of Interest

The authors declare no conflicts of interest.

Asthma was defined as physician-diagnosed asthma and ≥1 episode of wheeze in the previous year. A severe asthma exacerbation was defined as ≥1 hospitalization, or ≥1 visit to the emergency department or urgent care for asthma that lead to treatment with systemic (oral, intramuscular, or intravenous) corticosteroids, or ≥1 course of systemic steroid for asthma. IgE to each of five allergens (Der p 1, Bla g 2, Fel d 1, Can f 1, Mus m 1) was determined using the UniCAP 100 system (Pharmacia & Upjohn, Kalamazoo, MI). For each allergen, an IgE ≥ 0.35 IU/ml was considered positive, and atopy was defined as ≥1 positive IgE to the allergens tested.

Plasma vitamin D (25–hydroxy-vitamin D) was measured using the Waters high-performance liquid chromatography system with tandem mass spectrophotometry (Water Corporation, Milford, MA). Fourteen cytokines (IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)) were measured in plasma samples using Bio-Plex Pro Human TH17 cytokine panel on the BioPlex HTF system (Bio-Rad Laboratories Inc., Hercules, CA). Assays were designed on magnetic beads in a capture-sandwich immunoassay format, with samples measured in duplicate and levels presented as pg/mL. Undetectable cytokine levels were assigned a constant (half the lowest limit of detection, see eTable 1). All cytokine levels were log10-transformed for data analysis.

Bivariate analyses were conducted using two-sample *t*-tests or chi-square tests. Linear regression was used for the multivariable analysis of vitamin D or vitamin D insufficiency and cytokine levels, which was conducted in all subjects, and separately in cases and control subjects. Logistic regression was used for the multivariable analysis of vitamin D insufficiency and severe asthma exacerbations, which was stratified *a priori* by atopic vs. non-atopic asthma. All multivariable models were adjusted for age, sex, household income (<$15,000 or ≥$15,000 per year, the median household income for Puerto Rico in 2008–2009), and second-hand smoke (SHS) exposure. Models for severe exacerbations were additionally adjusted for body mass index (BMI) z-score (4), use of inhaled corticosteroid (ICS) in the prior six months, and four principal components (PCs) for the fourteen cytokines tested. Principal component analysis (PCA) was used to reduce the dimensionality of the 14 cytokines to a smaller set of uncorrelated independent components. PCs were extracted using varimax rotation, with the factor selection based on an eigenvalue cutoff of 1.0. SAS version 9.3 (SAS Institute, Inc, Cary, NC) was used for all analyses.

The main characteristics of study participants are summarized in eTable 2. Compared to children without asthma (n=283), those with asthma (n=295) were more likely to be male and to be currently exposed to SHS, and had a slightly higher vitamin D level. There were no significant differences in age, household income, type of health insurance, or vitamin D insufficiency between cases and control subjects.

Table 1 shows the results of the multivariable analysis of the relation between plasma vitamin D and log10-transformed cytokine levels. After a Bonferroni correction for 14 tests (cytokines), vitamin D was significantly and positively associated with IL-10 in all subjects ($P<0.0036$). Among all subjects, vitamin D was positively associated with IL-21, IL-25 and IL-31 at $P<0.01$. Similar results with regard to the magnitude and direction of the observed

associations were obtained in the analysis stratified by case-control status, but there was no significant result (likely due to smaller sample size). Similar findings were also obtained in an analysis of vitamin D insufficiency and cytokine levels (eTable 3).

Table 2 shows the multivariable analysis of vitamin D insufficiency and severe asthma exacerbations or hospitalizations for asthma, by atopic status. After adjusting for age, sex, household income, BMI z-score and use of ICS, vitamin D insufficiency was significantly associated with fivefold increased odds of ≥1 severe exacerbation among children with non-atopic asthma (**Model 1**). Similar findings were obtained after the multivariable models were additionally adjusted for levels of all plasma cytokines that were associated with vitamin D at $P<0.01$ in all subjects (IL-10, IL-21, IL-25 and IL-31; **Model 2**) or for the four cytokine-derived principal components (**Model 3**). Vitamin D insufficiency was similarly associated with increased odds of ≥1 hospitalization among children with non-atopic asthma (both models). In contrast to our results for non-atopic asthma, vitamin D insufficiency was less strongly and mostly non-significantly associated with severe asthma exacerbations or hospitalizations among children with atopic asthma.

Our findings further support detrimental effects of vitamin D insufficiency on severe asthma exacerbations through non-atopic mechanisms, as our results were largely unchanged after adjustment for plasma cytokines. For example, vitamin D may protect against viral or bacterial infections by strengthening epithelial barriers (5), or by increasing expression of antimicrobial peptides such as cathelicidin (6, 7). Alternatively, vitamin D may enhance response to ICS (8), as suggested by our finding of a positive association between vitamin D and IL-10 among all participants in the current study.

Our study is cross-sectional, and we cannot thus examine temporal relationships. Although we measured IgEs to five common allergens in Puerto Rico, we may have underestimated sensitization to other allergens. Moreover, we cannot exclude confounding by unmeasured variables, such as air pollution. Nonetheless, our findings suggest that vitamin D insufficiency leads to severe asthma exacerbations in children through non-atopic mechanisms, including resistance to ICS and enhanced susceptibility to viral infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Declaration of Funding

This work was supported by grants HL079966, HL117191, and HL119952 from the U.S. National Institutes of Health (NIH), and by The Heinz Endowments. Dr. Forno’s contribution was supported by grant HL125666 from the U.S. NIH.

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Clinical Implications

Our findings suggest that vitamin D insufficiency influences the pathogenesis of severe asthma exacerbations through non-atopic mechanisms, such as resistance to inhaled corticosteroids and enhanced susceptibility to viral infections.

Table 1

Multivariable analysis of the relation between plasma vitamin D level (predictor) and log10-transformed cytokine levels (outcomes), in all subjects and by case/control status

	All subjects (n=578)	Controls (n=283)	Cases (n=295)
Cytokine	**Coefficient (β)**	**Coefficient (β)**	**Coefficient (β)**
IL-1b	0.008	0.007	0.009
IL-4	0.010	0.013	0.008
IL-6	0.006	0.008	0.005
IL-10	**0.018***	0.018	0.018
IL-17A	−0.004	−0.007	−0.001
IL-17F	−0.001	0.015	−0.012
IL-21	0.012≠	0.010	0.014
IL-22	0.012	0.005	0.016
IL-23	0.006	0.002	0.009
IL-25	0.006≠	0.007	0.005
IL-31	0.014≠	0.019	0.010
IL-33	0.005	0.006	0.004
IFN-γ	0.006	0.015	−0.001
TNF-α	0.003	0.003	0.003

Multivariable linear models adjusted for age, sex, household income, current exposure to second-hand smoke, and (in all subjects) case/control status

≠P<0.01;

*P<0.0036 (0.05/14 tests)

Table 2

Vitamin D insufficiency (plasma 25(OH)D <30 ng/mL) and severe disease exacerbations in the prior year, by atopic vs. non-atopic asthma

	Vitamin D insufficiency		
	Model 1	**Model 2**	**Model 3**
	Odds ratio (95% confidence interval)		
Non-atopic asthma (n=92)			
≥ 1 severe asthma exacerbation	**5.0 (1.6 to 15.3)**‡	**5.6 (1.8 to 17.7)**‡	**5.8 (1.8 to 18.4)**‡
≥ 1 hospitalization for asthma	**5.2 (1.5 to 17.4)**‡	**5.2 (1.5 to 18.1)**‡	**6.2 (1.7 to 22.5)**‡
Atopic asthma (n=202)			
≥ 1 severe asthma exacerbation	1.9 (0.9 to 3.7)	**2.2 (1.1 to 4.4)**†	1.9 (0.9 to 3.7)
≥ 1 hospitalization for asthma	2.1 (0.9 to 4.7)	2.2 (0.9 to 5.0)	2.0 (0.9 to 4.4)

All models adjusted for age, sex, household income, BMI z-score and use of inhaled corticosteroids.

Model 2 additionally adjusted for plasma levels of IL-10, IL-21, IL-25, and IL-31.

Model 3 additionally adjusted for 4 principal components from cytokine analysis.

†P<0.05;

‡P<0.01