Bronchial Reactivity, Inflammatory and Allergic Parameters, and Vitamin D Levels in Children With Asthma

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BACKGROUND: A low serum vitamin D level may represent a marker of other perplexing factors that may lead to increased asthma prevalence and severity. Our aim was to assess the correlation between vitamin D levels and asthma and allergy markers in a subgroup of children with fewer confounding factors. METHODS: Non-obese children (6–18 y old) with asthma who were not receiving anti-inflammatory treatment were recruited. Subjects underwent spirometry with a methacholine challenge test, and fractional exhaled nitric oxide (F_{ENO}), serum vitamin D levels, total immunoglobulin E (IgE) levels, blood eosinophil counts, and high-sensitivity C-reactive protein levels were determined. The primary end point was the correlation between vitamin D level and airway hyper-responsiveness as assessed by a methacholine challenge test. The secondary end point was the correlation between vitamin D level and F_{ENO}, systemic inflammatory markers, and allergy. RESULTS: Seventy-one children with asthma (25 females, 35%; 12.5 ± 3.6 y of age) were included. The median vitamin D level was 23 ng/mL (range of 6–48.5, mean of 23.02 ± 7.74), the median IgE level was 305 IU/mL (range of 4.3–4,240), the median provocational concentration of methacholine that produced a 20% decrease in FEV_1 was 1.1 mg/mL (range of 0–13.9), and the median F_{ENO} was 26.5 ppb (range of 3.6–285). No correlation was found between vitamin D level and response to the methacholine challenge test, F_{ENO}, high-sensitivity C-reactive protein levels, IgE levels, eosinophil counts, and frequency of allergic rhinitis or atopic dermatitis. CONCLUSIONS: In our group of children with asthma, no correlation was found between the level of vitamin D and the degree of airway reactivity, airway inflammation, and allergy. The cause-and-effect relationship between vitamin D, asthma, and allergy should be further clarified. (ClinicalTrials.gov registration NCT01287455) *Key words: vitamin D; airway hyper-responsiveness; fractional exhaled NO; high-sensitivity C-reactive protein; asthma; allergic parameters.* [Respir Care 0;0(0):1–•. © 0 Daedalus Enterprises]

Introduction

Asthma is a chronic inflammatory disorder characterized by increased airway hyper-responsiveness (AHR) and variable air-flow obstruction.[1] The inflammatory process in asthma is assumed to be a T helper cell type 2 (Th2)-driven process that enhances eosinophil accumulation and immunoglobulin (IgE) production, leading to AHR.[2,3] Asthma prevalence is rising in both the developed and the developing world, with an estimation of > 300 million people affected worldwide.[4,5]

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Many potential factors and reasons have been suggested to lead to the increasing prevalence of asthma; it has been proposed that vitamin D deficiency may explain a portion of the asthma epidemic. Vitamin D is a preprohormone that has 2 parent forms: cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). Cholecalciferol is synthesized in human skin from its precursor upon exposure to sunlight and may also be ingested in the diet (primarily from eating oily fish). Ergocalciferol is the plant and fungal form of the vitamin, which may occasionally be ingested in the diet primarily by eating fungi. Vitamin D deficiency is one of the most prevalent medical conditions and is spreading worldwide.[6] Indeed, previous studies reported a high burden of vitamin D deficiency even in sun-replete areas.[7,8] Case-control studies addressing the interconnection between vitamin D and asthma showed that children with asthma had a higher prevalence of vitamin D deficiency compared with control subjects.[9,10] Moreover, epidemiologic studies implicated the role of vitamin D deficiency in allergy and asthma development and exacerbations.[11-14] In a large cohort of Costa Rican children with asthma, an inverse relation between the serum vitamin D levels and the total IgE levels and eosinophil counts was demonstrated.[15] Other studies have shown that low serum vitamin D levels are associated with reduced lung function in adolescents,[16] poor asthma control,[17] and the presence of exercise-induced bronchoconstriction in children with asthma.[18] A recent study showed increased levels of vitamin D-binding protein and 25-hydroxyvitamin D in bronchoalveolar lavage fluid after allergen challenge, suggesting a role for these factors in allergic late-phase reactions.[19]

A low serum vitamin D level may represent a marker of Western life style. Modern life is associated with less time spent outdoors; altered nutrition; increased obesity; exposure to cigarette smoking, ultrafine particles, and ozone; and inadequate physical activity. These factors are linked to increased asthma prevalence and increased severity. Non-obese children with asthma who are not receiving anti-inflammatory drugs may represent a specific subgroup with fewer confounding factors. The aim of this study was to examine the relationship between serum vitamin D levels and AHR, fractional exhaled nitric oxide (F_{ENO}), high-sensitivity C-reactive protein levels, and allergy markers in a specific subgroup of children with asthma.

Methods

Study Population

Children (6–18 y old) with controlled or partially controlled asthma as defined by Global Initiative for Asthma guidelines[1] were included if they were not receiving preventive anti-inflammatory drugs (>2 weeks), bronchodilators (>24 h), or systemic corticosteroids (>2 months). Exclusion criteria were: obesity defined by body mass index above the 85th percentile for age and sex[20]; any chronic illness, respiratory infection, or emergency room visit 2 months before study initiation; acute illness; or $FEV_1 < 65\%$ on the study day.

Demographics and personal and family history of respiratory disease, allergic symptoms, and smoking were recorded. Spirometry with a methacholine challenge test was performed, and F_{ENO}, serum vitamin D levels, total IgE levels, peripheral blood eosinophil counts, and high-sensitivity C-reactive protein levels were evaluated. This study was approved by the Rambam Medical Center institutional review committee (number 483-10) and registered at the National Institutes of Health (NCT01287455). All parents signed an informed consent before their children entered the study.

Vitamin D Status

A single measurement of serum vitamin D concentration (measured as 25-hydroxyvitamin D) was performed using a radioimmunoassay method. Values were categorized as sufficient (> 30 ng/mL), insufficient (20–30 ng/mL), and deficient (< 20 ng/mL) on the basis of previous recommendations.[21]

Pulmonary Function Testing

Spirometry was conducted in accordance with American Thoracic Society recommendations[22] using a KoKo spirometer (nSpire Health, Longmont, Colorado). The best FEV_1 and FVC were selected for data analysis. Subjects

were instructed to avoid using short-acting and long-acting bronchodilators for at least 6 and 24 h, respectively, before evaluation. Results were expressed as percent of predicted derived from Quanjer et al.[23]

Methacholine Challenge Test

Subjects whose FEV_1 was at least 65% of predicted on baseline spirometry underwent methacholine challenge testing at the same visit. The methacholine challenge test was performed according to American Thoracic Society guidelines using triple concentrations of methacholine (0.057–13.95 mg). The solutions were administered using a dosimeter (nSpire Health). Nebulized methacholine was inhaled for 2 min, with 5-min intervals between doses, until the maximal concentration or end point was reached. The provocational concentration of methacholine that produced a 20% decrease in FEV_1 (PC_{20}) was determined. On completion of the methacholine challenge test, 200 mg of albuterol via an inhaler with a spacer device was given to all subjects to restore airway caliber.[24]

F_{ENO}

The subjects performed 3 online single-breath maneuvers according to international guidelines[25] using an Eco Medics NO analyzer (CLD 88sp F_{ENO} analyzer, Eco Physics, Dürnten, Switzerland). Blood samples were analyzed for complete blood counts and serum IgE, high-sensitivity C-reactive protein, and vitamin D levels.

Statistical Analysis

The primary end point was defined as the correlation between vitamin D levels and the degree of AHR as assessed by a methacholine challenge test in this cohort of children. The secondary end point was defined as the correlation between F_{ENO}, systemic markers of inflammation and allergy, and vitamin D levels.

The data were evaluated using SPSS 18 (SPSS, Chicago, Illinois). The Kolmogorov-Smirnov test was used to assess normal distribution of the variables. Logarithmic transformation of the data was done whenever the distribution was highly skewed. The Mann-Whitney U test, t test, and analysis of variance were applied to compare differences between vitamin D levels and numerical parameters. Pearson correlation was used for quantitative variables. A chi-square test was performed to determine correlation between categorical variables (sex, smoking, season, allergy). $P < .05$ was considered significant. A sample size of at least 20 children in each group was calculated to detect a 1-mg difference in PC_{20} between the group with vitamin D levels < 30 ng/mL and the group with vitamin D levels > 30 ng/mL (2-sided 95% CI, power of 80%).

Table 1. Subject Characteristics

Characteristic	Subjects ($N = 71$)
Age, y	12.5 ± 3.6
Female, n (%)	25 (35)
BMI, kg/m^2	20.0 ± 3.6
Vitamin D, ng/mL	23.02 ± 7.74
Total IgE, IU/mL	305 (4.3–4,240)
Eosinophil count, cells/μL	0.38 (0–4.3)
FEV_1, L	2.2 (1–4.2)
FEV_1, % predicted	89 ± 11
FEV_1/FVC	0.8 (0.6–1)
$FEF_{25-75\%}$, L/s	2.2 (0.8–5.3)
$FEF_{25-75\%}$, % predicted	81 ± 20
F_{ENO}, ppb	26.5 (3.6–285)
C-reactive protein, mg/L	1.7 (0.9–7.7)
PC_{20}, mg/mL	1.1 (0–13.9)
Allergic rhinitis, n (%)	38 (53.5)
Atopic dermatitis, n (%)	10 (14.3)

Values are given as median (interquartile range) or mean ± SD unless indicated otherwise.
BMI = body mass index
IgE = immunoglobulin E
$FEF_{25-75\%}$ = forced expiratory flow during the middle half of the FVC maneuver
F_{ENO} = fractional exhaled nitric oxide
PC_{20} = provocational concentration of methacholine that produced a 20% decrease in FEV_1

Results

Seventy-one children with asthma referred to our Pediatric Pulmonology Unit at Meyer Children's Hospital of Rambam Health Care Campus were included in the study. All participants live in the Haifa district, in the north part of the country. The main characteristics of the subjects are reported in Table 1. All subjects had positive methacholine challenge tests ($PC_{20} < 13.95$ mg). Twelve subjects (16.9%) had sufficient vitamin D levels (> 30 ng/mL), 33 subjects (46.5%) had insufficient levels (20–30 ng/mL), and 26 subjects (36.6%) had deficient levels (< 20 ng/mL) ($P < .001$). The mean body mass index of the subjects was $20.0 ± 3.6$ kg/m^2 (< 75th percentile and < 65th percentile for boys and girls, respectively). Allergic rhinitis was reported in 38 subjects (53.5%), and IgE levels of > 30 IU/mL were found in 62 subjects (87.3%). No active smoking was reported within the study population.

We did not find any correlation between serum vitamin D levels and FEV_1, PC_{20}, F_{ENO}, eosinophil counts, and IgE levels in our study population (Fig. 1). When stratified to subjects with $FEV_1 \leq 80\%$ of predicted ($n = 15$) and subjects with $FEV_1 > 80\%$ of predicted ($n = 56$), similar vitamin D levels were observed ($P = .55$). Similarly, subjects categorized according to the degree of bronchial responsiveness as moderate or severe (methacholine challenge test ≤ 1 mg, $n = 33$), mild (methacholine challenge test ≤ 4 mg, $n = 24$), and borderline (methacholine challenge test ≥ 4 mg, $n = 14$) had similar vitamin D levels.
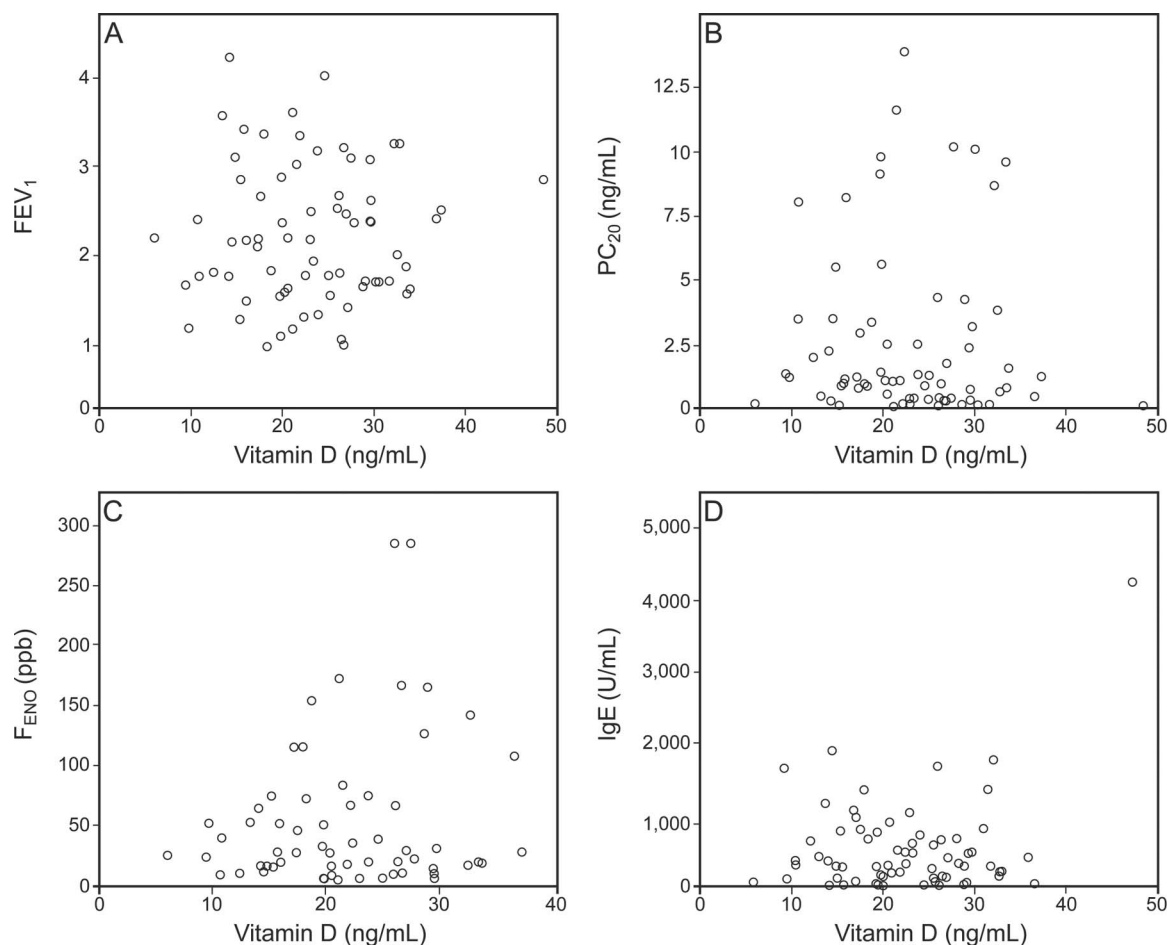
Fig. 1. Scatter plots of the relationship between serum vitamin D levels and FEV_1 (A), provocational concentration of methacholine that produced a 20% decrease in FEV_1 (PC_{20}) (B), fractional exhaled nitric oxide (F_{ENO}) (C), and immunoglobulin E (IgE) levels (D). No correlation between vitamin D levels and the stated parameters was found (Pearson correlation with logarithmic transformation of the data whenever the distribution was highly skewed).

Table 2 shows the main characteristics of the subjects stratified by vitamin D levels. Only 12 subjects had sufficient vitamin D levels. As shown by subgroup analysis according to vitamin D levels, no statistically significant difference in FEV_1, PC_{20}, F_{ENO}, IgE levels, and eosinophil counts was found between children with vitamin D deficiency compared with children with insufficient and sufficient vitamin D levels. Subanalysis of the vitamin D-deficient group revealed no correlation between vitamin D and F_{ENO}, IgE levels, PC_{20}, and FEV_1 (correlation coefficient of 0.13, $P = .52$; -0.48, $P = .82$; 0.29, $P = .15$; -0.18, $P = .39$, respectively).

Discussion

In our group of children with asthma, no correlation between reduced serum vitamin D levels and lung function, AHR, F_{ENO}, IgE levels, eosinophil counts, or C-reactive protein levels was found. Assuming that vitamin D deficiency is directly related to proliferation of Th2 cells,[26,27] lower vitamin D levels should be related to AHR, increased F_{ENO}, IgE levels, and eosinophilia.

Pulmonary Function Tests

In contrast to previous studies,[9-18,28-31] we found no correlation between serum vitamin D levels and pulmonary function. Our results are supported by studies in adults with asthma[32] and studies that failed to show an association between vitamin D levels and pulmonary function, degree of asthma control, or doses of inhaled corticoids in children with asthma.[33]

AHR

Only a few studies have assessed the association between vitamin D deficiency and AHR. A study of 54 adults (24 on inhaled steroids) showed that reduced serum vita-

Table 2.   Subgroup Analysis According to Vitamin D Levels

Parameter	Vitamin D Levels			P
	< 20 ng/mL	20–30 ng/mL	> 30 ng/mL	
Subjects, n (%)	26 (36.6)	33 (46.5)	12 (16.9)	
Age, y	3.6 ± 12.9	3.6 ± 12.5	11.6 ± 3.9	.59
Females, n (%)	8 (31)	14 (42)	3 (25)	.46
BMI, kg/m^2	21.2 ± 4.4	19.4 ± 3.0	19.2 ± 2.5	.12
Vitamin D, ng/mL	15.18 ± 3.65	25.06 ± 3.02	34.44 ± 4.93	< .001
Total IgE, IU/mL	361.5 (16–1,890)	260.5 (4.3–1,680)	433.5 (37.4–4,220)	.06
Eosinophil count, cells/μL	0.35 (0.0–0.9)	0.40 (0.0–4.3)	0.42 (0.2–1)	.46
FEV$_1$, L	2.2 (1–4.2)	2.22 (1–4)	1.95 (1.6–3.3)	.99
FEV$_1$, % predicted	90 ± 13.7	89 ± 9.8	85 ± 7.5	.39
FEV$_1$/FVC	0.81 (0.6–1)	0.82 (0.6–1)	0.83 (0.8–1)	.17
FEF$_{25-75\%}$, L/s	1.8 (0.8–5.3)	2.20 (0.8–4.6)	2.42 (1.5–3.1)	.89
FEF$_{25-75\%}$, % predicted	83 ± 17.3	81 ± 23.8	78 ± 15	.78
F$_{ENO}$, ppb	29.3 (4.7–153)	23.7 (3.6–285)	22.9 (16–142)	.59
C-reactive protein, mg/mL	1.73 (0.9–6.2)	1.57 (0.9–4.8)	1.72 (1.1–7.7)	.34
PC$_{20}$, mg/mL	1.33 (0.1–9.8)	0.86 (0.1–13.9)	0.99 (0.1–10.1)	.55
Allergic rhinitis, n (%)	12 (46.2)	20 (60.6)	6 (50)	.52
Atopic dermatitis, n (%)	3 (11.5)	4 (12.5)	3 (25)	.52

Values are given as median (interquartile range) or mean±SD unless indicated otherwise.
BMI = body mass index
FEF$_{25-75\%}$ = forced expiratory flow during the middle half of the FVC maneuver
F$_{ENO}$ = fractional exhaled nitric oxide
PC$_{20}$ = provocational concentration of methacholine that produced a 20% decrease in FEV$_1$

min D concentration was associated with greater AHR.[34] A study comparing 45 children with asthma with 59 healthy control subjects found that children with asthma and exercise-induced bronchospasm had lower vitamin D levels.[18] No correlation between AHR and serum vitamin D levels was observed in our cohort of 71 children with asthma. It should be noted that multivariate analysis performed in a very large cohort study in Costa Rican children with asthma failed to show a statistically significant inverse association of serum vitamin D with airway responsiveness.[15] In addition, a recent study evaluating subjects with hereditary 1,25-dihydroxyvitamin D-resistant rickets (lacking the vitamin D receptor) showed protection against methacholine challenge test-provoked bronchial hyper-reactivity.[35]

F$_{ENO}$

In our study, we failed to show any significant association between vitamin D levels and F$_{ENO}$ in children with asthma. This result is supported by a large population sample (1,315 children) that failed to demonstrate significant association between serum 25-hydroxyvitamin D levels and F$_{ENO}$,[29] and a recent interventional study that failed to demonstrate an effect of vitamin D administration on F$_{ENO}$ levels.[36] It should be noted that although F$_{ENO}$ is

considered to be a marker of eosinophilic inflammation, its value is still under debate.

C-reactive protein is a sensitive inflammatory marker that is synthesized in hepatocytes. Previous population-based studies showed a correlation between increased levels of high-sensitivity C-reactive protein and a high frequency of AHR and low FEV$_1$.[37] No study has evaluated C-reactive protein in relation to serum vitamin D levels in children with asthma. No relationship between vitamin D levels and C-reactive protein was observed in our study.

No association between serum vitamin D levels and IgE levels or eosinophil counts was observed in our cohort of children with asthma. Our results contradict those of a previously published study showing an inverse relationship between serum vitamin D levels and total IgE levels or eosinophil counts.[15] However, 2 recent studies showed similar results.[38,39] We suggest that these differences may be due to the small sample size and selection of subjects with milder asthma compared with subjects with more severe asthma in the other studies.

A putative explanation for the association observed between severe asthma and allergy and low serum vitamin D levels is that patients with severe asthma are more likely to spend more time indoors with less sun exposure, leading to lower serum vitamin D levels. Additionally, asthma severity is related to socioeconomic status and parental educa-

tion. Higher socioeconomic status may be related to a higher intake of all vitamin and nutrient supplements that could modify asthma severity, such as vitamin E supplementation. A recent study from Turkey showed that children with asthma had less exposure to sunlight and ate a diet less rich in vitamin D compared with healthy control children.[40] In a recent systematic review of cohort studies concerning the association between low vitamin D levels and asthma in children, 3 cohort studies were eligible: 2 studies reported positive association, and the third found no association.[41] The high prevalence of vitamin D deficiency (36.6%) and insufficiency (46.5%) in our group of children with asthma is in agreement with previous studies.[10,15] However, it should be noted that the frequency of vitamin D deficiency was not assessed in a healthy control population residing in the same area.

Although most reports suggest a role for vitamin D in asthma control, others show that vitamin D may promote, rather than ameliorate, the asthma phenotype.[42,43] These differences have also been reported regarding the effects of vitamin D on Th2 cell responses. One study addressing this issue reported that vitamin D supplementation through the first year of life (2,000 IU/d) elicited an increased rather than decreased risk of developing atopy, allergic rhinitis, and asthma in adulthood.[44] Two recent interventional studies with vitamin D supplementation failed to show improvement in asthma control.[36,45]

When vitamin D values were categorized as sufficient ($>$ 30 ng/mL), insufficient (20–30 ng/mL), and deficient ($<$ 20 ng/mL), similar results were observed. Of note, serum vitamin D cutoff levels were determined for bone health use, whereas in nonbone-related conditions, there are still no optimal levels for disease prevention. Specifically in asthma and allergy, researchers are still trying to define the serum levels of vitamin D that can ameliorate and prevent asthma exacerbations.

Limitations

The frequency of vitamin D deficiency was not assessed in a healthy control group. We chose children with controlled or partially controlled asthma who were not receiving treatment to perform methacholine challenge tests and to avoid the possible impact of anti-inflammatory drugs or severe asthma on vitamin D levels. Our results may be related to selection of children with asthma with few symptoms and fewer confounding factors. Alternatively, controlled or partially controlled asthma patients may have a genetic polymorphism in the vitamin D receptor that may be associated with better asthma control.[46]

Although it was a relatively large cohort, the sample size was still small and included only 12 subjects with sufficient vitamin D levels, thus limiting the statistical power. Skin tests were not performed, and allergy was assessed by history, IgE levels, and eosinophil counts. The small number of subjects with $FEV_1 \leq 80\%$ of predicted may have precluded statistically significant change. Long-term follow-up studies focusing on changes in vitamin D status and asthma parameters are needed to clarify the effect of vitamin D status on asthma.

Conclusions

In summary, our study supports previous findings of a high prevalence of vitamin D deficiency in children with asthma. However, no correlation between vitamin D levels and response to a methacholine challenge test, F_{ENO}, high-sensitivity C-reactive protein levels, IgE levels, and eosinophil counts was found in our cohort of children with asthma. These results emphasize the complexity of the effects of vitamin D on the immune system and its potential role in asthma prevalence and pathophysiology. More high-quality studies are needed to reliably clarify this issue, and well-designed randomized controlled trials of interventional vitamin D studies assessing supplementation with vitamin D or placebo in asthma are also needed.

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