

Vitamin D Insufficiency and Asthma Severity in Adults From Costa Rica

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Purpose: Non-classical actions of vitamin D as a cytokine are related to the immunopathology of asthma. Few studies have examined vitamin D levels and asthma severity in adults. The aim of this research was to assess the relationship between vitamin D levels, atopy markers, pulmonary function, and asthma severity. **Methods:** We analyzed 25-hydroxyvitamin D levels in serum collected from 121 asthmatic adults from Costa Rica to investigate the association between vitamin D levels (categorized as sufficient, ≥ 30 ng/mL, or insufficient, < 30 ng/mL), allergic rhinitis, total IgE and peripheral blood eosinophils (as markers of atopy), asthma severity, baseline forced expiratory volume in 1 second (FEV1), and forced vital capacity (FVC). Univariate and multivariate analyses were performed to assess these relationships. **Results:** When the population was stratified by vitamin D status, 91% of asthmatic patients with vitamin D levels below 20 ng/mL ($n=36$) and 74% of patients with vitamin D levels between 20 and 30 ng/mL ($n=73$) had severe asthma versus 50% of those with vitamin D sufficiency ($n=12$; $P=0.02$). Vitamin D insufficiency was associated with a higher risk of severe asthma (odds ratio [OR], 5.04; 95% Confidence interval [CI], 1.23-20.72; $P=0.02$). High vitamin D levels were associated with a lower risk of hospitalization or emergency department visit during the last year (OR, 0.90; 95% CI, 0.84-0.98; $P=0.04$). Although there appeared to be a direct relationship between vitamin D levels and FEV1 (regression coefficient=0.48; $r^2=0.03$), it did not reach statistical significance ($P=0.07$). **Conclusions:** Our findings suggest that vitamin D insufficiency is common among our cohort of asthmatic adults. Lower vitamin D levels are associated with asthma severity.

Key Words: Adult; asthma; vitamin D

INTRODUCTION

The incidence of asthma has been increasing globally, though mainly in Westernized and industrialized nations, and there is a trend of higher prevalence farther away from the equator. Asthma chronicity, morbidity, and mortality represent a major public issue in several regions due to their high prevalence.¹ Moreover, recent epidemiological studies suggest that the prevalence of vitamin D insufficiency is also increasing in some countries due to dietary and behavioral changes over the last few decades.²

It has been suggested that there is a link between this high prevalence of asthma and vitamin D deficiency. In support of this hypothesis, two studies have shown that vitamin D supplementation might prevent the development of asthma and improve the clinical response to steroids.^{3,4} Vitamin D deficiency has been associated with increased airway hyperresponsiveness, lower pulmonary function, worse asthma control, and

steroid resistance.⁵ It has been demonstrated that insufficient vitamin D levels (defined by serum concentrations < 30 ng/mL) were associated with an increased risk of severe asthma during childhood.⁶ Furthermore, a study carried out in asthmatic children from Costa Rica showed that high serum vitamin D levels were associated with reduced risk of any hospitalization in the previous year, lower use of anti-inflammatory medications for asthma, and less airway hyperresponsiveness.⁷

Vitamin D has been shown to have a role in both innate and

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adaptive immunity, by promoting phagocytosis and modulating the effects of Th1, Th2, and regulatory T cells.^{8,9} Further evidence suggests that vitamin D alters human airway smooth muscle expression of chemokines and inhibits the expression of a steroid resistant gene.¹⁰

The majority of these studies were carried out in children, and this association has not been well-studied in adults. Moreover, the prevalence of vitamin D insufficiency is unknown in Costa Rica, a tropical country located 11° 13' north and 08° 02' south with abundant sun exposure throughout the year, resulting in a lack of seasons and high ultraviolet index.

The aim of the current study was to assess the relationship between 25-hydroxyvitamin D, atopy markers (total IgE, eosinophil count, allergic rhinitis), the risk of severe asthma, hospitalization or Emergency Department (ED) visit due to asthma during the last year, and pulmonary function (through the assessment of forced expiratory volume in 1 second [FEV1], and forced vital capacity [FVC]) in 121 asthmatic adults from Costa Rica.

MATERIALS AND METHODS

Study subjects

One-hundred and twenty-one consecutive patients older than 18 years from the Pneumology Department of the San Juan de Dios Hospital (San José, Costa Rica) were included in the study if they had a pneumologist-confirmed diagnosis of asthma according to the Global Initiative for Asthma (GINA) criteria. Definition of severe asthma was done by the pneumologist in charge if the patient had partly controlled or uncontrolled asthma (according to the level of control proposed by GINA) during the last 4 weeks before serum vitamin D was determined, without regard to optimal treatment, adherence, or inhalation technique.¹¹

A high steroid dose was defined as a patient having received more than 1,000 µg per day of inhaled beclomethasone dipropionate (chlorofluorocarbon) or any oral glucocorticoid.

Data and blood samples were collected between July 1, 2011 and August 30, 2011. Patient medical information was obtained via the medical record. Patients were excluded if they had used vitamin D or calcium supplements in their diet. Approval of this protocol was received from the Institutional Review Board, and written informed consent was obtained from all participants.

Serum 25-Hydroxyvitamin D3

Blood samples were collected during the examination. Serum levels of 25-hydroxyvitamin D3 (hereafter referred to as vitamin D) were assayed using an enzyme immunoassay kit (Immuno-diagnostic Systems, Scottsdale, AZ, USA). This is considered the best circulating biomarker of vitamin D metabolic status because it reflects total vitamin D from dietary intake and sunlight exposure as well as the conversion of vitamin D from adipose tissue in the liver.^{11,12} We categorized vitamin D levels as sufficient (≥ 30 ng/mL) or insufficient (<30 ng/mL) based on previous recommendations.¹³

Total IgE and eosinophil count

Total serum IgE levels were determined using an automated chemiluminescent immunometric assay (Immulite Total IgE 1000; Siemens, Erlangen, Germany). Peripheral blood eosinophil counts were conducted using fluorescent flow cytometry using an automated hematology analyzer (XT-1800i; Sysmex, Mundelein, IL, USA). All analyses were performed by the Nuclear Medicine and Clinical Laboratories at San Juan de Dios Hospital.

Table 1. Patients' characteristics.

Characteristic	All patients (n=121)	Vitamin D level			P value
		≥ 30 ng/mL (n=12)	20-30 ng/mL (n=73)	<20 ng/mL (n=36)	
Age (yr)	48.1 ± 15.7	42.4 ± 13.5	46.1 ± 16.4*	54.1 ± 13.8*†	0.02
Female sex (%)	101 (83.5)	10 (83.3)	60 (82.2)	31 (86.1)	0.87
BMI ≥ 30 kg/m ² (%)	50 (41.32)	4 (33.33)	30 (41.11)	16 (44.4)	0.79
Any hospitalization or ED visit (%) [‡]	20.7	8.3	17.8	44.4	0.004
FEV1 (% predicted)	72.9 ± 18.3	81.6 ± 14.5	73.7 ± 18.4	68.4 ± 18.5	0.08
FEV1/FVC ratio	69.7 ± 5.4	71.2 ± 4.9	68.6 ± 5.7	69.9 ± 5.3	0.53
IgE (IU/mL)	788.8 ± 3,588.8	257.7 ± 220.9	4,586 ± 536.8	721 ± 120.3	0.64
Eosinophil count (# cells/mL)	254.4 ± 255.4	198.8 ± 110.0	283.6 ± 296.9	215.9 ± 189.6	0.33
Allergic rhinitis (%)	78.50	91.70	76.70	77.78	0.54
Severe asthma [†] (%)	76.70	50.0	74.0	91.4	0.02
High steroid dose (%)	84.30	94.40	80.80	75.00	0.23

* $P < 0.05$, compared to ≥ 30 ng/mL.

† $P < 0.05$, compared to 20-30 ng/mL.

‡ $P < 0.05$, chi-square test.

BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.

Spirometry

Spirometry was conducted in accordance with the American Thoracic Society (ATS) recommendations¹⁴ using a Cardinal Health flow-screen spirometer (Dublin, OH, USA). The best predicted FEV1 and FEV1/FVC values were selected for our analysis. Reference values were obtained from the NHANES III (Third National Health and Nutrition Examination Survey) registry according to weight, height, ethnicity, age, and gender, as recommended by the ATS. All analyses were performed in the morning.

Statistical analysis

Descriptive analyses and unadjusted between-group comparisons were performed using one-way ANOVA and *post hoc* tests when the variables were continuous and had equal variances or using Chi-square (linear by linear correlation) when they were categorical. Univariate relationships are presented in Table 1. Variables were considered statistically significant at *P* values of less than 0.05 using two-sided tests.

Two multivariate models were constructed by logistic regression analysis. A stepwise approach was performed to build these models. We summarized overall calibration using a Hosmer-Lemeshow goodness-of-fit test.

We conducted a retrospective analysis relating asthma severity as a binary outcome and baseline vitamin D levels categorized as sufficient or insufficient. We adjusted for potential confounders, including age, sex, body mass index (BMI), total IgE, high

Table 2. Multivariate analysis of potential risk factors for severe asthma.

Covariate	Odds ratio	95% Confidence interval	<i>P</i> value
FEV1 (% predicted)	0.98	(0.95-1.00)	0.38
IgE (IU/mL)	1.00	(0.99-1.00)	0.76
BMI (kg/m ²)	1.02	(0.96-1.12)	0.62
Age (yr)	0.95	(0.92-0.98)	0.01
Male sex	1.92	(0.59-6.17)	0.27
Vitamin D insufficiency	5.04	(1.23-20.72)	0.02
High steroid dose	3.23	(0.87-11.97)	0.08

FEV1, forced expiratory volume in 1 sec; BMI, body mass index.

Table 3. Multivariate analysis of potential risk factors for hospitalization or ED visit due to asthma during the past year.

Covariate	Odds ratio	95% Confidence interval	<i>P</i> value
FEV1 (% predicted)	0.99	(0.97-1.02)	0.98
Vitamin D (ng/mL)	0.90	(0.84-0.98)	0.04
BMI (kg/m ²)	1.05	(0.98-1.13)	0.38
Age (yr)	0.99	(0.96-1.03)	0.69
High steroid dose	3.72	(0.47-29.90)	0.21
Male sex	1.57	(0.40-6.15)	0.52

FEV1, forced expiratory volume in 1 sec; BMI, body mass index.

steroid use, and FEV1. Another retrospective analysis was carried out with a logistic regression procedure relating any hospitalization or ED visit during the last year and vitamin D levels (as a continuous variable) adjusted for potential pre-established confounders including age, sex, BMI, high steroid dose, and FEV1. We also studied the relationship between vitamin D levels and FEV1 (as continuous variables) using a simple linear regression model. All analyses were performed using SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

The baseline characteristics of the overall population stratified by vitamin D sufficiency status are shown in Table 1. A statistically significant relationship was found between asthma severity and vitamin D sufficiency status: 91.4% of the patients with vitamin D levels below 20 ng/mL and 74.0% of the patients with vitamin D levels between 20 and 30 ng/mL had severe asthma in comparison with 50% of the patients with vitamin D sufficiency (*P*=0.02). There was also a non-significant trend towards lower FEV1 in patients with insufficient vitamin D levels (*P*=0.08). We did not observe any association between FEV1/FVC and vitamin D status.

Vitamin D insufficiency as a predictor of the risk of severe asthma and any hospitalization or visit to the ED

Multivariate analysis of risk factors for severe asthma is shown in Table 2. The odds of having severe asthma were 5.04 times higher in patients with insufficient vitamin D levels than in those who had sufficient vitamin D levels (*P*=0.02). We also observed in this model that aging was associated with a lower risk of severe asthma (*P*=0.01). As it is depicted in Table 3 and Figure,

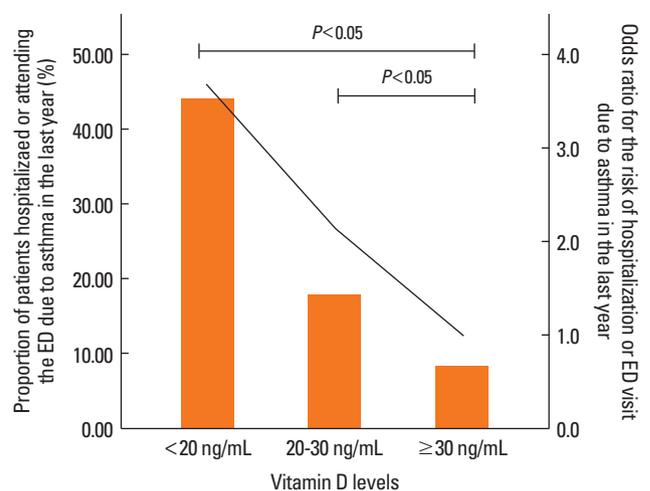


Figure. Proportion of patients (bars) and odds ratio, compared to subjects with vitamin D levels ≥ 30 ng/mL (line), for any hospitalization or ED visit due to asthma during the previous year according to vitamin D level.

multivariate analysis showed a 10% reduction in the odds of any hospitalization or ED visit due to asthma during the last year for each 1 ng/mL increase in vitamin D level ($P=0.04$).

Vitamin D level and lung function

Serum vitamin D levels were not significantly correlated with FEV1 in the linear regression analysis. According to this model, there was an increase of 0.49% in the predicted FEV1 for each 1 ng/mL increase in vitamin D (regression coefficient=0.49; $r^2=0.028$; $P=0.07$).

DISCUSSION

In this report we demonstrate a high prevalence of vitamin D insufficiency among asthmatic adults in Costa Rica (latitude, 10°N). This percentage (90%) is higher than that reported in another study of Costa Rican asthmatic children, in which 28% had vitamin D insufficiency,⁷ and much higher than the 20% reported by Alyasin et al.¹⁵ in Iranian children. However, this high percentage is similar to that reported by Li et al.¹⁶ in asthmatic adults from China and is consistent with the epidemics of hypovitaminosis D in developing countries reported by Arabi et al.¹⁷

Besides, it has been reported that in a sunny region, dress habits, sunscreen use, sunlight avoidance, and other behavioral factors can provoke hypovitaminosis D, even in otherwise healthy individuals.¹⁸ Indeed, a previous study carried out in Puerto Rico, located on a similar geographical latitude as Costa Rica (18° 15' north), reported a high prevalence of vitamin D insufficiency in children with (44%) and without (47%) asthma.¹⁹

The discrepancies between our findings, reporting such a high prevalence of vitamin D insufficiency, and other studies,^{14,20} can be attributed mainly to behavioral variables. Female sex and being overweight are well-known risk factors for a low level of vitamin D,²¹ although we did not find any relationship between these two variables and vitamin D level in our cohort. Furthermore, inherited factors may explain why some populations living at the same latitude but with a distinct genetic background exhibit different vitamin D levels, for example, as a consequence of genetic polymorphisms involved in cholesterol synthesis, hydroxylation, and vitamin D transport.²²

We did not find any association between vitamin D levels and some allergy markers (eosinophil count, total IgE, allergic rhinitis). Such associations have been reported by others only inconsistently. Some studies have revealed a clear association between vitamin D level and these markers of atopy,^{4,7} while others reported no relationship.^{6,22} These contradictory results can be explained in part by the different populations, the variability of ultraviolet exposure and vitamin D level throughout the year in other latitudes, and the different exposure to allergens in other regions. Therefore, these findings must be regarded cautiously because the small sample size, the experimental design, and

some other confounding variables could bias our results. Moreover, it has been demonstrated that the relationship between vitamin D and IgE is nonlinear,²³ and this marker of atopy can lead to misleading interpretations in settings of very low vitamin D levels. Besides, we did not assay some factors (such as allergen-specific IgE antibodies, IL-4, or IL-5), which can be more specific for assessment of atopy in asthmatic patients.²⁴

Overall, the main finding of this study was the significant association between vitamin D levels, the risk of severe asthma, and the risk of hospitalization or visit to the ED due to asthma. These findings are consistent with prior evidence that children with low vitamin D levels have increased asthma severity, more exacerbations, and a concomitant need for escalating pharmacologic intervention.^{4,6}

Vitamin D can affect the physiopathology of asthma through several mechanisms. Vitamin D plays a critical role in innate and adaptive immunity by activating antimicrobial peptides, like cathelicidin.^{8,25} This peptide is known to be active against a wide range of bacteria, viruses, mycobacteria, and fungi, and its deficiency is related to a higher susceptibility to infection and asthma exacerbations.²⁵ Vitamin D also inhibits the synthesis and release of Th1-associated cytokines and some other molecules, like IL-17, thereby leading to decreased inflammation and smooth muscle cell proliferation.^{5,9} Furthermore, recent studies have shown that reduced vitamin D levels are associated with increased expression of the pro-inflammatory cytokine-TNF- α , enhancing a pro-inflammatory effect in asthmatic patients.²⁶ This vitamin promotes regulatory T cells and also increases synthesis of IL-10, leading to an inhibition of Th2 responses as well as airway inflammation and airway hyperresponsiveness.²⁷

Also, even if we did not categorize vitamin D levels according to the cutoff of 30 ng/mL, we still found a significant relationship between vitamin D level as a continuous variable and the risk of hospitalization or ED visit as an indicator of asthma severity. This finding further supports the concept that there remains insufficient data to determine an optimal vitamin D level for all the effects of this hormone besides calcium homeostasis. Although previous reports have demonstrated an association between inhaled corticosteroid, oral corticosteroid use, and total steroid dose with lower vitamin D levels,⁴ we did not find any relationship between these two variables, mainly because there was a high prevalence of high steroid dose among all the participants.

In our study, linear association analysis of serum vitamin D levels and FEV1 revealed a trend towards a linear relationship between these two variables. This finding suggests the involvement of vitamin D in lung function, as previously reported by others.^{4,18,28} Vitamin D inhibits the formation of matrix metalloproteinases as well as fibroblast and smooth muscle cell proliferation.²⁹

There are some biases that may have influenced the interpre-

tation of our results. Firstly, reverse causation (severe asthma leading to vitamin D insufficiency because of limited physical activity and time outdoors) cannot be adequately excluded in a cross-sectional study such as this. Selection bias is likely to occur if there was differential entry into the study for reasons related to the exposure (vitamin D) and the outcome (severe asthma). Moreover, there was an unbalanced female-to-male ratio in our study, and for this reason our findings are not necessarily representative of Costa Rican adults with asthma.

In conclusion, we demonstrated that vitamin D insufficiency is frequent in asthmatic adults and that it is predictive of the risk of severe asthma. Low vitamin D levels also predict the odds of hospitalization or ED visit due to asthma, and it seems to be associated with decreased lung function. We suggest a randomized clinical trial to determine whether supplementation with vitamin D can reduce or modify asthma severity in adults.

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