









## Original Article

# Serum Vitamin D Is Inversely Associated with Blood Eosinophil Count Among Adults with Allergic Asthma

Dante Daniel Hernández-Colín<sup>1</sup> , Martín Bedolla-Barajas<sup>1</sup> , Jaime Morales-Romero<sup>2</sup> ,  
Martín Robles-Figueroa<sup>3</sup> , Angie Bedolla-Pulido<sup>4</sup> , Nora del Rosario Arroyo-Sánchez<sup>3</sup> 

<sup>1</sup>Department of Allergy and Immunology, Nuevo Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”, Guadalajara, México

<sup>2</sup>University of Veracruzana, de Salud Pública Institute, Xalapa, México

<sup>3</sup>Department of Internal Medicine, Nuevo Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”, Guadalajara, México

<sup>4</sup>University of de Guadalajara Faculty of Health Sciences, Guadalajara, México

**Cite this article as:** Hernández-Colín DD, Bedolla-Barajas M, Morales-Romero J, Robles-Figueroa M, Bedolla-Pulido A, del Rosario Arroyo-Sánchez N. Serum vitamin D is inversely associated with blood eosinophil count among adults with allergic asthma. *Thorac Res Pract.* 2023;24(4):208-213.

## Abstract

**OBJECTIVE:** To evaluate the role that serum vitamin D concentration plays in the number of eosinophils in peripheral blood in adults with allergic asthma.

**MATERIAL AND METHODS:** A total of 142 patients were categorized based on different cutoff points for eosinophils:  $\geq 200$ ,  $\geq 300$ ,  $\geq 400$ , and  $\geq 500$  cells/mL. The vitamin D concentration was stratified into  $< 20$  and  $\geq 20$  ng/mL. The association between vitamin D (independent variable) and eosinophils (dependent variable) was explored using multivariate analysis.

**RESULTS:** The average number of eosinophils in the included patients was 418 cells/mL, and 33.8% of the included patients had vitamin D concentrations  $\geq 20$  ng/mL. Asthmatic patients with vitamin D  $< 20$  ng/mL had a higher mean concentration of eosinophils than did asthmatic patients with vitamin D  $\geq 20$  ng/mL ( $464 \pm 377.7$  eosinophils/mL vs.  $327.8 \pm 247.2$  eosinophils/mL,  $P = .025$ ). We also observed that vitamin D was inversely correlated with eosinophil count ( $\rho = 0.244$ ,  $P = .003$ ). In the multivariate analysis, vitamin D  $< 20$  ng/mL showed a significant inverse association with each cutoff value for eosinophilia (odds ratio  $> 1$ ).

**CONCLUSION:** Vitamin D concentrations  $< 20$  ng/mL are associated with a significant increase in the number of eosinophils in blood. Studies that analyze the use of vitamin D supplements as complementary therapy for the treatment of asthma are needed.

**KEYWORDS:** Allergy, asthma, eosinophils, vitamin D

**Received:** August 21, 2022

**Accepted:** March 15, 2023

**Publication Date:** July 21, 2023

## INTRODUCTION

Among the most frequent hypovitaminosis worldwide is vitamin D (VD) deficiency. In the adult population, the prevalence of VD deficiency ( $< 20$  ng/mL) is approximately 40%.<sup>1-3</sup> Vitamin D is mostly recognized for its influence on calcium and phosphorus metabolism as well as bone metabolism; however, an increasing number of extraosseous effects are increasingly drawing attention. 25-hydroxyvitamin D (25[OH]D) (calcitriol), the active form of VD, participates in the regulation of innate and adaptive immune responses. It favors the production of defensins and cathelicidins and improves chemotaxis and the function of physical barriers, among other things. In the adaptive immune response, calcitriol inhibits the production of Th1 cytokines (IL-12, IFN- $\gamma$ , IL-6, IL-8, TNF- $\alpha$ , and IL-9) and increases the production of anti-inflammatory cytokines Th2 (IL-4, IL-5, and IL-10); in addition, it inhibits the proliferation of Th1 cells and induces the production of Treg cells.<sup>4</sup> At the level of eosinophils, VD reduces necrosis and, consequently, the secondary release of cytotoxic granules from eosinophils in tissues.<sup>5</sup> Eosinophils cultured in a VD-free medium exhibit spontaneous activation; in contrast, the levels of activation products are reduced in the presence of VD.<sup>6</sup>

In Latin America, there is little information on the relationship between VD and blood eosinophils in adults with asthma. Our primary objectives were, first, to determine the prevalence of alterations in serum VD concentration and, second, to evaluate the role of serum VD concentration on the number of eosinophils in peripheral blood.

## MATERIAL AND METHODS

### Study Site and Patients

Our teaching hospital is located in the metropolitan area of Guadalajara in western Mexico. In this metropolitan area, the average elevation is 1540 m above sea level; the climate is temperate most of the year, with an average temperature of 18°C; and the months with the greatest solar radiation are from March to August, with a pronounced peak during May.<sup>7</sup>

In this cross-sectional study, only adults aged 18 years or older were included, and all were residents of the metropolitan area. Patients with a history of respiratory disease exacerbated by aspirin or near-fatal asthma, those who were using

**Corresponding author:** Martín Bedolla-Barajas, e-mail: drmbdobar@gmail.com



VD supplements, systemic steroids, anticonvulsants, rifampicin, or antiretrovirals during the previous 4 weeks, and those who had an asthma attack 1 month prior to the study were not included. The patient inclusion period was from October 2017 to April 2019.

### Serum Vitamin D, IgE, and Eosinophils

Vitamin D, total serum IgE, and eosinophils in peripheral blood were quantified in the central laboratory of the hospital on the same day that the blood sample was obtained. The magnetic particle-based chemiluminescence technique was used to quantify VD (AccessTotal®, Beckman Coulter) and total IgE (AccessTotal IgE®, Beckman Coulter) concentrations; the former is expressed in ng/mL, and the latter is expressed in IU/mL. Flow cytometry (CELL-DYN Ruby™; Abbott Diagnostics Division, Abbott Laboratories, IL, USA) was used to quantify the total eosinophil concentration, expressed as cells per microliter (μL).

### Skin Testing and Forced Spirometry

Allergic sensitization was determined by the presence of at least 1 positive skin test to 1 or more of the tested aeroallergens (house dust mites, dander, cockroach mix, pollen from grasses, trees and weeds, and fungal spores). Histamine was used as a positive control, and glycerin was used as a negative control. The aeroallergens were placed on both volar surfaces of the forearms, and a Hollister Stier® lancet was used for puncture; skin test results were interpreted in accordance with international guidelines.<sup>8</sup>

Respiratory function (forced expiratory volume [FEV<sub>1</sub>] and forced vital capacity [FVC]) was assessed via spirometry using a Master Screen-Body PFT spirometer (Jaeger®, CareFusion, Baesweiler, Germany). Each participant performed a maximum of 8 maneuvers or until 3 tests met the criteria of acceptability and reproducibility. In addition, a challenge test with a short-acting bronchodilator inhaler (salbutamol 0.4 mg) was performed. This test served to assess the reversibility of airway obstruction.

### Definitions

The following cutoff points were used to classify eosinophilia in blood:  $\geq 200$ ,  $\geq 300$ ,  $\geq 400$ , and  $\geq 500$  cells/μL.

Serum VD concentrations  $\geq 20$  ng/mL were considered sufficient to maintain calcium homeostasis.<sup>9</sup>

Serum levels of total IgE  $\geq 100$  IU/mL were considered outside the normal range.

Allergic asthma was considered the presence of symptoms compatible with the disease, in addition to evidence of reversible airway obstruction and at least 1 positive skin test to one of the tested aeroallergens.

### Ethics

Our study was approved by the ethics committee of the New Civil Hospital of Guadalajara "Dr. Juan I. Menchaca"; each of the patients had to sign a written informed consent form to participate in the study, another to undergo spirometry, and another to undergo skin prick tests.

### Statistical Analyses

Quantitative variables with a normal distribution are presented as the mean and SD. Categorical variables are presented as frequencies and proportions. The Spearman rho correlation test was performed to detect an association between the variables. Finally, to identify variables associated with eosinophilia, different multivariate logistic regression analysis models were performed, 1 for each eosinophilia cutoff value. A value of  $P < .05$  was considered statistically significant. Data analysis was performed with the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp.; Armonk, NY, USA).

### RESULTS

In this study, a total of 142 patients were included (75% were women), and the mean age was 35 years (Table 1). The frequencies of allergic rhinitis and atopic dermatitis in patients with asthma were 82% and 10%, respectively; 37% had a personal history of hospitalization for asthma during the previous year. Based on the results of asthma control tests (ACT), 28% of the subjects perceived their asthma as controlled. Mites and cockroaches were the allergens to which patients were most sensitized, with atmospheric fungi being the allergen to which patients were least sensitized. Regarding respiratory function, the mean baseline FVC and FEV<sub>1</sub> were 88% and 71% of the predicted value. Notably, asthmatics with VD concentrations  $< 20$  ng/mL had significantly lower FVC and FEV<sub>1</sub> than patients with VD greater than  $\geq 20$  ng/mL. The mean total serum IgE concentration was 418 IU/mL; 67% of the patients had concentration equal to or greater than 100 IU/mL. The mean VD concentration was  $18.4 \pm 8.1$  ng/mL, and in 33.8% of the patients, the concentration was  $\geq 20$  ng/mL. The mean and median absolute blood eosinophil counts were 418 cells/μL and 340 cells/μL, respectively. When comparing the median and mean concentration of eosinophils between patients with asthma and VD  $< 20$  ng/mL and patients with VD  $\geq 20$  ng/mL, there was a statistically significant difference, i.e., 359 eosinophils/mL vs. 240 eosinophils/μL ( $P = .023$ ) and  $464 \pm 378$  eosinophils/μL vs.  $327.8 \pm 247$  eosinophils/μL ( $P = .025$ ), respectively.

The following provides the prevalence of eosinophilia in blood based on the different cutoff values: eosinophils  $\geq 200$  cells/μL:  $n = 107$  (75.4%, 95% CI 67.6% to 81.7%); eosinophils  $\geq 300$  cells/μL:  $n = 78$  (54.9%, 95% CI 46.7% to 62.9%); eosinophils  $\geq 400$  cells/μL:  $n = 58$  (40.8%, 95% CI 33.1% to

#### MAIN POINTS

- The frequency of vitamin D concentration  $\geq 20$  ng/mL in patients with asthma was 33.8%.
- The prevalence of eosinophilia in blood based on the different cutoff values were: eosinophils  $\geq 200$  cells/μL, 75.4%; eosinophils  $\geq 300$  cells/μL, 54.9%; eosinophils  $\geq 400$  cells/μL, 40.8%; and, eosinophils  $\geq 500$  cells/μL, 30.3%.
- Asthmatic patients with vitamin D concentration  $< 20$  ng/mL had higher blood eosinophil concentrations than patients with asthma and vitamin D  $\geq 20$  ng/mL.
- There was an inverse association between vitamin D concentration and blood eosinophils.

**Table 1.** Characteristics of the Population with Asthma

	Total (n = 142)	Vitamin D		P
		<20 ng/mL (n = 94)	≥20 ng/mL (n = 48)	
Age (years), mean ± SD	34.6 ± 10.4	36.0 ± 10.2	31.8 ± 10.3	.024
Sex, female, n (%)	106 (74.6)	69 (73.4)	37 (77.1)	.634
Current smoking, n (%)	8 (5.6)	6 (6.4)	2 (4.2)	.717
Alcohol consumption, n (%)	33 (23.2)	18 (19.1)	15 (31.3)	.106
Atopic comorbidity, n (%)				
Allergic rhinitis	117 (82.4)	74 (78.7)	43 (89.6)	.108
Food allergy	27 (19.0)	18 (19.1)	9 (18.8)	.954
Atopic dermatitis	13 (9.2)	8 (8.5)	5 (10.4)	.709
Urticaria	7 (4.9)	2 (2.1)	5 (10.4)	.044
BMI (kg/m <sup>2</sup> ), mean ± SD	29.1 ± 6.2	29.8 ± 6.7	27.9 ± 4.9	.078
Hospitalization in the previous year, n (%)	52 (36.6)	32 (34.0)	20 (41.7)	.372
ACT, points				
Mean ± SD	15.2 ± 5.4	14.6 ± 5.4	16.2 ± 5.3	.103
≥20, n (%)	40 (28.2)	24 (25.5)	16 (33.3)	.328
Allergic sensitization, n (%)				
Mites	94 (66.2)	60 (63.8)	34 (70.8)	.404
Cockroaches	70 (49.3)	45 (47.9)	25 (52.1)	.635
Trees	67 (47.2)	43 (45.7)	24 (50.0)	.631
Epithelia	57 (40.1)	37 (39.4)	20 (41.7)	.791
Weeds	60 (42.3)	36 (38.3)	24 (50.0)	.182
Grass	43 (30.3)	29 (30.9)	14 (29.2)	.836
Fungi	18 (12.7)	12 (12.8)	6 (12.5)	.964
Respiratory function, mean ± SD				
% predicted baseline				
FVC	87.9 ± 18.3	85.1 ± 18.9	93.4 ± 16.0	.011
FEV <sub>1</sub>	71.2 ± 19.2	68.8 ± 20.55	75.9 ± 15.4	.039
FEV <sub>1</sub> /FVC	67.1 ± 10.3	66.7 ± 11.2	67.9 ± 8.1	.493
Total serum IgE, IU/mL				
Median (P <sub>25</sub> -P <sub>75</sub> )	211 (74-502)	204 (67-524)	224 (82-501)	.626
IgE ≥100, n (%)	95 (66.9)	61 (64.9)	34 (70.8)	.477
Vitamin D, ng/mL				
Mean ± SD	18.4 ± 8.1	14.2 ± 3.7	26.5 ± 8.2	<.0001
≥20, n (%)	48 (33.8)	N/A	N/A	N/A
Eosinophils, cells/μL				
Mean ± SD	418.1 ± 344.5	464 ± 378	328 ± 247	.025
Median (P <sub>25</sub> -P <sub>75</sub> )	340 (205-580)	359 (227-602)	240 (145-477)	.023

ACT, asthma control test; BMI, body mass index; FEV<sub>1</sub>, forced expiratory volume; FVC, forced vital capacity; IgE, immunoglobulin E; N/A, not applicable; P<sub>25</sub>-P<sub>75</sub>, percentile 25-percentile 75.

49.1%); and, eosinophils ≥500 cells/μL: n = 43 (30.3%, 95% CI 23.3% to 38.3%).

As seen in the correlation matrix shown in Table 2, VD concentration was inversely correlated with blood eosinophil count ( $P = .003$ , statistical power of 84%) and age ( $P = .037$ ). Blood eosinophil count was inversely correlated with age

( $P = .0398$ ) and ACT ( $P = .006$ ) and positively correlated with IgE ( $P = .002$ ). Additionally, there was a significant correlation between age with body mass index (BMI) (positive correlation), FEV<sub>1</sub> (negative correlation), and IgE (negative correlation) ( $P < .0001$ ). Asthma control test was significantly correlated with FEV<sub>1</sub>. There was no significant correlation between VD and BMI, FEV<sub>1</sub>, ACT, and IgE. There was also

**Table 2.** Magnitude of Correlation Between Variables

	Eosinophils	Vitamin D	Age	BMI	FEV <sub>1</sub>	ACT	IgE
Eosinophils	1						
Vitamin D	-0.244 <i>P</i> = .003	1					
Age	-0.071 <i>P</i> = .398	-0.176 <i>P</i> = .037	1				
BMI	-0.104 <i>P</i> = .217	-0.048 <i>P</i> = .570	0.317 <i>P</i> < .0001	1			
FEV <sub>1</sub>	-0.067 <i>P</i> = .428	0.096 <i>P</i> = .258	-0.298 <i>P</i> < .0001	-0.053 <i>P</i> = .513	1		
ACT	-0.230 <i>P</i> = .006	0.028 <i>P</i> = .743	-0.017 <i>P</i> = .842	-0.087 <i>P</i> = .305	0.427 <i>P</i> < .0001	1	
IgE	0.255 <i>P</i> = .002	0.070 <i>P</i> = .411	-0.317 <i>P</i> < .0001	-0.145 <i>P</i> = .086	0.088 <i>P</i> = .300	-0.160 <i>P</i> = .057	1

Correlation estimated through Spearman's rho.

ACT, asthma control test; BMI, body mass index; FEV<sub>1</sub>, forced expiratory volume in the first second, predicted baseline; IgE, immunoglobulin E.

no significant correlation between eosinophils and BMI and FEV<sub>1</sub>. There was no significant correlation between the other variables.

Table 3 presents several multivariate models of factors associated with blood eosinophilia based on the following cutoff points:  $\geq 200$ ,  $\geq 300$ ,  $\geq 400$ , and  $\geq 500$  cells/ $\mu\text{L}$ . In all adjusted models, VD  $< 20$  ng/mL exhibited a statistically significant inverse association with eosinophilia. In contrast, IgE  $\geq 100$  IU/mL was identified as a risk factor for eosinophilia for all cutoff points, except when the eosinophil count was  $\geq 500$  cells/ $\mu\text{L}$ . Other risk factors for eosinophilia were sex (female) with an eosinophil count  $\geq 400$  cells/ $\mu\text{L}$  and fungal sensitization with an eosinophil count  $\geq 500$  cells/ $\mu\text{L}$ . Last, age younger than 35 years was inversely associated with an eosinophil count  $\geq 500$  cells/ $\mu\text{L}$ .

## DISCUSSION

In this study, many patients with asthma showed alterations in VD concentration. When we compared the number of eosinophils between asthmatic patients with VD  $< 20$  ng/mL and those with VD  $\geq 20$  ng/mL, the former had a noticeably higher concentration of eosinophils in peripheral blood.

Based on the results of this study, VD deficiency in patients with allergic asthma is not infrequent, as up to 70% had VD concentrations lower than 20 ng/mL. This result contrasts markedly with those from population studies. In the United States, the National Health and Nutrition Examination Survey 2005-2006 showed that slightly more than 0% of the analyzed population had VD levels  $< 20$  ng/dL<sup>3</sup>; similar findings were observed in Mexico, 43.6% of adults also had VD levels  $< 20$  ng/dL.<sup>1</sup> There are different reasons to explain why VD may be insufficient in this population, including low exposure to sunlight, skin color, age, use of sunscreen, and low physical activity.<sup>10</sup> In our study, most patients had moderate airway obstruction, which may have contributed to less outdoor physical activity; consequently, they would be less exposed to ultraviolet radiation, thus favoring less VD

production. The use of systemic or topical steroids at high doses can also contribute to this same problem.

Notably, in adult patients with allergic asthma and VD deficiency, there was an inverse association with eosinophil count in peripheral blood. This finding is consistent with previous studies conducted in children and adults with allergic and nonallergic asthma.<sup>11,12</sup> The use of VD supplements in asthmatic patients with VD deficiency has been shown to significantly reduce allergic inflammation.<sup>2</sup> Similar to asthmatic patients, patients with allergic rhinitis and VD deficiency also have increased eosinophil concentrations in blood compared with that in controls.<sup>13</sup> Last, the results herein indicate that a cutoff point of VD  $\geq 20$  ng/mL serves as a good predictor of eosinophilia in blood when the eosinophil count is 200 cells/ $\mu\text{L}$  or more. The modulating role of VD in the immune response seems to be responsible for its effect on eosinophils by favoring an increase in the production of Th2 cytokines in addition to inhibiting the proliferation of Th1 cells.<sup>14</sup> According to The Global Initiative for Asthma, VD supplementation may reduce the number of asthma exacerbations or improve asthma control, especially when VD levels are  $< 20$  ng/mL.<sup>13</sup>

Among other relevant findings in this study is the positive association between IgE concentration and eosinophils in blood. Notably, the relationship was stronger when the amount of eosinophils was  $\geq 200$  or  $\geq 300$  cells/ $\mu\text{L}$ , slightly diminished at  $\geq 400$  cells/ $\mu\text{L}$ , and finally disappeared at  $\geq 500$  cells/ $\mu\text{L}$ . A wide network of cytokines, including IL-4, IL-5, IL9, IL-13 and IL-31, is involved in the regulation of IgE expression and eosinophils; however, IL-4 and IL-13 are the main cytokines involved in T2 inflammation.<sup>15</sup> In our study population, all asthmatic patients were sensitized to allergens; thus, different mechanisms that favor eosinophil expression or fungal sensitization, as we present below, are probably involved in the lack of an association between IgE and eosinophil quantities  $\geq 500$  cells/ $\mu\text{L}$ . Although IgE is a biomarker of T2 inflammation, it has been suggested that IgE concentration does not help identify patients with asthma who will respond better to treatment with type 2 monoclonal antibodies<sup>15</sup>; therefore,

**Table 3.** Different Multivariate Models to Identify Factors Associated with Eosinophilia in Patients with Asthma

Dependent variable: eosinophils $\geq 200$ cells/ $\mu$ L			
	aOR	95% CI	P
Vitamin D <20 ng/mL	2.84	1.29-6.26	.020
Female	—	—	.361
Age $\geq 35$ years	—	—	.441
Allergic rhinitis	—	—	.981
ACT $\geq 20$ points	—	—	.578
IgE $\geq 100$ IU/mL	3.18	1.40-7.21	.006
Sensitization to fungi	—	—	.144
Dependent variable: eosinophils $\geq 300$ cells/ $\mu$ L			
	aOR	95% CI	P
Vitamin D <20 ng/mL	2.62	1.53-6.56	.002
Female	—	—	.227
Age $\geq 35$ years	—	—	.448
Allergic rhinitis	—	—	.806
ACT $\geq 20$ points	—	—	.067
IgE $\geq 100$ IU/mL	3.55	1.67-7.57	.001
Sensitization to fungi	—	—	.896
Dependent variable: eosinophils $\geq 400$ cells/ $\mu$ L			
	aOR	95% CI	P
Vitamin D <20 ng/mL	2.20	1.01-4.79	.046
Female	3.22	1.32-7.82	.010
Age $\geq 35$ years	—	—	.133
Allergic rhinitis	—	—	.268
ACT $\geq 20$ points	—	—	.431
IgE $\geq 100$ IU/mL	2.92	1.32-6.49	.008
Sensitization to fungi	—	—	.318
Dependent variable: eosinophils $\geq 500$ cells/ $\mu$ L			
	aOR	95% CI	P
Vitamin D <20 ng/mL	3.16	1.27-7.85	.013
Female	—	—	.096
Age $\geq 35$ years	0.40	0.18-0.89	.025
Allergic rhinitis	5.33	1.36-20.93	.016
ACT $\geq 20$ points	—	—	.147
IgE $\geq 100$ IU/mL	—	—	.377
Sensitization to fungi	3.65	1.19-11.15	.023

OR obtained by binary logistic regression. All variables were entered dichotomously. The OR was not calculated for those variables that were not included in the model because they did not present a statistically significant association.  
 ACT, asthma control test; aOR, adjusted odds ratio; IgE, immunoglobulin E.

eosinophils in blood become a better therapeutic target for the control of asthma.

In this study, an eosinophil count  $\geq 500$  cells/ $\mu$ L was 60% less frequent in patients with asthma and was only observed in

those over 35 years old. The emergence of biological products for the control of T2 asthma redefined the role of eosinophils as a therapeutic target, marking the need to define the best cutoff points to achieve the greatest benefit of their use. Thus, there are some biologics that are indicated when the eosinophil count is  $\geq 150$  cells/ $\mu$ L and others that are indicated when the count is  $\geq 400$  cells/ $\mu$ L.<sup>16</sup> In our study population, 75% of patients had eosinophil counts  $\geq 200$  cells/ $\mu$ L, but only 30% had counts  $\geq 500$  cells/ $\mu$ L. It seems that with increasing age, the number of eosinophils decreases, as do their effector functions.<sup>17</sup>

We also observed that allergic rhinitis in asthmatic patients was significantly associated with blood eosinophilia; however, this occurred only when eosinophil counts were  $\geq 500$  cells/ $\mu$ L. The mechanisms that could explain this phenomenon are not clear, but it has been shown that the concentration of eosinophils in blood is higher when allergic rhinitis is not controlled than when it is controlled.<sup>18</sup>

Finally, in our study, slightly more than 10% of the patients were sensitized to fungi; this finding is consistent with previously published data.<sup>19,20</sup> Notably, this event was a factor associated with blood eosinophilia, especially when the eosinophil count was  $\geq 500$  cells/ $\mu$ L. Colonization by fungi in patients with asthma is recognized for its ability to induce vigorous immune responses, which justifies the intentional search for allergic bronchopulmonary mycosis in patients with allergic sensitization to fungi; for this, new diagnostic criteria have been recently published.<sup>21</sup>

Due to the design characteristics of the study, we were not able to establish if the association between VD and eosinophilia is constant; it has been shown that the levels of eosinophils in blood can vary throughout the day and that, when treating allergic patients, they can be modified by exposure to sensitizing allergens. However, having used different cutoff points to define eosinophilia, it is unlikely that the association remained stable. Another limitation is the use of topical or systemic steroids by some participants at the time of inclusion in the study and the potential effect on blood eosinophils. Notably, we were careful not to include those patients with a history of systemic steroid use 1 month before participants were selected for this study. Regarding the use of inhaled corticosteroids, the patients were not using them regularly; but once the diagnosis of asthma was established, they were instructed to use them regularly and at adequate doses. Finally, we did not investigate the presence of parasitosis concomitant with asthma, diet characteristics, or the use of solar radiation blockers, all factors likely to modify the association between the variables.

**CONCLUSION**

In summary, several patients with asthma have VD concentrations below 20 ng/mL (more than 70%). In this study, VD levels <20 ng/mL were associated with a greater number of total eosinophils in peripheral blood in adults with allergic asthma. These findings fully justify the analysis of the repercussions of VD deficiency at the eosinophil level, i.e., eosinophil count, and the functional level. Similarly, more research that analyzes the use of VD supplements as complementary therapy for the treatment of asthma is needed.

**Ethics Committee Approval:** This study was approved by Ethics committee of New Civil Hospital of Guadalajara "Dr. Juan I. Menchaca" (Approval No: CGI/JDI/042/2017).

**Informed Consent:** Written informed consent was obtained from the patients who agreed to take part in the study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – M.B.B., A.B.P, N.R.A.S.; Design – M.B.B., J.M.R., N.R.A.S.; Supervision – M.B.B., D.D.H.C., M.R.F.; Resources – D.D.H.C., M.R.F., A.B.P.; Materials – M.B.B., A.B.P, N.R.A.S.; Data Collection and/or Processing – M.B.B., A.B.P, N.R.A.S.; Analysis and/or Interpretation – M.B.B., J.M.R., D.D.H.E.N.; Literature Search – M.B.B., A.B.P, N.R.A.S.; Writing – M.B.B., J.M.R.; Critical Review – M.B.B., D.D.H., J.M.R., M.R.F., A.B.P, N.R.A.S.

**Declaration of Interests:** The authors have no conflict of interest to declare.

**Funding:** This study received no funding.

## REFERENCES

- Clark P, Vivanco-Muñoz N, Piña JT, et al. High prevalence of hypovitaminosis D in Mexicans aged 14 years and older and its correlation with parathyroid hormone. *Arch Osteoporos*. 2015; 10:225. [\[CrossRef\]](#)
- de Groot JC, van Roon EN, Storm H, et al. Vitamin D reduces eosinophilic airway inflammation in nonatopic asthma. *J Allergy Clin Immunol*. 2015;135(3):670-5.e3. [\[CrossRef\]](#)
- Parva NR, Tadepalli S, Singh P, et al. Prevalence of vitamin D deficiency and associated risk factors in the US population (2011-2012). *Cureus*. 2018;10(6):e2741. [\[CrossRef\]](#)
- Bui L, Zhu Z, Hawkins S, Cortez-Resendiz A, Bellon A. Vitamin D regulation of the immune system and its implications for COVID-19: A mini review. *SAGE Open Med*. 2021;9: 20503121211014073. [\[CrossRef\]](#)
- 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(2):119-129. [\[CrossRef\]](#)
- Lu H, Xie RD, Lin R, et al. Vitamin D-deficiency induces eosinophil spontaneous activation. *Cell Immunol*. 2017;322:56-63. [\[CrossRef\]](#)
- Ulloa H, García M, Meulenert A, et al. El clima y potencial solar en la zona metropolitana de Guadalajara, Jalisco, México. Perspectivas de uso energético. Accessed May 7, 2021. <http://www.ommac.org/congreso2011/document/extenso/Ext2011041.pdf>.
- van Kampen V, de Blay F, Folletti I, et al. EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies. *Allergy*. 2013;68(5):580-584. [\[CrossRef\]](#)
- IOM (Institute of Medicine). *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: The National Academies Press; 2011.
- Cashman KD. Vitamin D deficiency: defining, prevalence, causes, and strategies of addressing. *Calcif Tissue Int*. 2020; 106(1):14-29. [\[CrossRef\]](#)
- Hollams EM, Hart PH, Holt BJ, et al. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur Respir J*. 2011;38(6):1320-1327. [\[CrossRef\]](#)
- Souto Filho JTD, de Andrade AS, Ribeiro FM, Alves PAS, Simonini VRF. Impact of vitamin D deficiency on increased blood eosinophil counts. *Hematol Oncol Stem Cell Ther*. 2018;11(1):25-29. [\[CrossRef\]](#)
- Wu HY, Chen JX, Tian HQ, Zhang XL, Bian HY, Cheng L. Serum 25-hydroxyvitamin D inversely associated with blood eosinophils in patients with persistent allergic rhinitis. *Asia Pac Allergy*. 2017;7(4):213-220. [\[CrossRef\]](#)
- Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention*. [www.ginasthma.org](http://www.ginasthma.org); 2022.
- Akdis CA, Arkwright PD, Brügggen MC, et al. Type 2 immunity in the skin and lungs. *Allergy*. 2020;75(7):1582-1605. [\[CrossRef\]](#)
- McGregor MC, Krings JG, Nair P, Castro M. Role of biologics in asthma. *Am J Respir Crit Care Med*. 2019;199(4):433-445. [\[CrossRef\]](#)
- Mathur SK, Schwantes EA, Jarjour NN, Busse WW. Age-related changes in eosinophil function in human subjects. *Chest*. 2008;133(2):412-419. [\[CrossRef\]](#)
- Novakova P, Staevska M, Novakova S, Mateva N. Association between blood eosinophil count and control of allergic rhinitis: does it exist? *Eur Respir J*. 2019;54:PA4400. [\[CrossRef\]](#)
- Bedolla-Barajas M, Hernández-Colín DD, Sainz-Hernández J, Morales-Romero J. Sensibilización a alérgenos en adultos mexicanos con asma; la experiencia en un hospital escuela. *Rev Alerg Mex*. 2011;58(3):133-141.
- Forkel S, Beutner C, Schröder SS, et al. Sensitization against fungi in patients with airway allergies over 20 years in Germany. *Int Arch Allergy Immunol*. 2021;182(6):515-523. [\[CrossRef\]](#)
- Asano K, Hebisawa A, Ishiguro T, et al. New clinical diagnostic criteria for allergic bronchopulmonary aspergillosis/mycosis and its validation. *J Allergy Clin Immunol*. 2021;147(4):1261-1268.e5. [\[CrossRef\]](#)