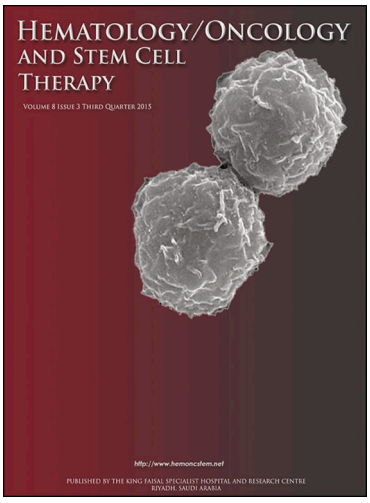

Original Research Report

Impact of Vitamin D Deficiency on Increased Blood Eosinophil Counts

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ORIGINAL ARTICLE

Impact of Vitamin D Deficiency on Increased Blood Eosinophil Counts

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Abstract

Objective/Background: Vitamin D has been increasingly recognized as an immunomodulatory agent. Its deficiency has been associated with immune-mediated diseases such as asthma, rhinitis, and atopic dermatitis. These allergic conditions are dependent on T-helper type 2 (Th2) cells secreting interleukins, overproduction of immunoglobulin E (IgE), and eosinophil activation. We investigated the association between serum vitamin D levels and blood absolute eosinophil count.

Methods: We carried out a cross-sectional study of 669 men and women referred to a clinical pathology laboratory who underwent 25-hydroxyvitamin D testing and complete blood count analysis on the same day.

Results: Vitamin D levels were stratified into four ranges: severely deficient (<10 ng/mL), deficient (≥10 ng/mL and <20 ng/mL), insufficient (≥20 ng/mL and <30 ng/mL), or sufficient (≥30 ng/mL). The mean/median eosinophil count in the four groups was 267/254 cells/μL, 245/238 cells/μL, 191/159 cells/μL, and 182/146 cells/μL, respectively,

(p = .001). The difference was significant between the severe deficiency group and each of the other three groups (p = .012, p = .002, and p = .001, respectively). There was no statistical difference among the four groups in terms of leukocyte counts (p = .151), neutrophils (p = .177), or lymphocytes (p = .582).

Conclusion: Vitamin D deficiency was associated with higher blood eosinophil count. These results support the possible role of vitamin D in the eosinophil immune response.

Keywords: eosinophilia; eosinophils; vitamin D; vitamin D deficiency

Running author: J.T.D.S. Filho et al.

Running title: Vitamin D Deficiency and Blood Eosinophil Counts

Introduction

Vitamin D plays an important role in calcium and bone metabolism as well as immunomodulation.[1] It is mostly generated in the skin by the conversion of provitamin D_3 to previtamin D_3 during exposure to sunlight, but some vitamin D is also derived from food sources. Vitamin D is converted in the liver to 25-hydroxyvitamin D—25(OH)D_3—and then converted to the active form, 1,25-dihydroxyvitamin D—1,25(OH)D_3—or calcitriol, in the kidneys.[2]

It is known that vitamin D is a fundamental immunoregulator that exerts extensive anti-inflammatory actions through the vitamin D receptor (VDR) expressed in the immune system.[1] The protective role of vitamin D against allergic diseases has been supported by associations between low serum 25(OH)D_3 levels and higher rates of asthma,[3–7] atopic dermatitis,[8,9] and allergic rhinitis.[10]

Eosinophils have long been associated with the effector arm of T-helper type 2 (Th2) cell immunity engaged in allergic responses.[11] However, a correlation between serum 25(OH)D_3 levels and circulating eosinophils is not strongly established. A few studies have noted that lower levels of vitamin D are associated with an increased blood eosinophil count,[3,12] but most other studies have reported no significant association.[4,5,7,13–16]

Given these uncertainties, we sought to evaluate the impact of serum 25(OH)D_3 deficiency on Th2 activation and increase circulating eosinophils in peripheral blood.

Materials and methods

This cross-sectional study was carried out on patients who had been referred to a medical laboratory on Campos dos Goytacazes, Rio de Janeiro, Brazil, from January to December 2015. Patients who underwent 25(OH)D_3 testing and complete blood count analysis on the same day were included in the study. We analyzed the following parameters: age, sex, leukocyte count, absolute eosinophil, neutrophil, and lymphocyte counts.

The study was reviewed and approved by the Institutional Ethics Committee (Faculty of Medicine of Campos, Campos dos Goytacazes, Rio de Janeiro, Brazil) and was conducted in accordance with the principles of the Declaration of Helsinki. In this study, institutional consent was obtained for record review, and all patient information was anonymized and deidentified prior to analysis.

25(OH)D_3 is the major circulating form of vitamin D, and its measurement is the best indicator of overall vitamin D status.[2] Peripheral venous blood samples were obtained from all patients and serum 25(OH)D_3 levels were measured using an electrochemiluminescence immunoassay. Serum 25(OH)D_3 levels were categorized as

severely deficient (<10 ng/mL), deficient (≥10 ng/mL and <20 ng/mL), insufficient (≥20 ng/mL and <30 ng/mL), or sufficient (≥30 ng/mL).

A peripheral smear was performed. After Wright–Giemsa staining, the percentage of eosinophil cells was computed relative to the total number of white blood cells. The absolute eosinophil count was computed by multiplying the total leukocyte count by the percentage of eosinophil cells. The total leukocyte count was measured using Coulter counter techniques.

Data description was primarily based on means ± standard deviations and medians for continuous end points and on frequencies for categorical end points. We used the nonparametric Kruskal–Wallis tests for the statistical analysis to compare continuous variables between independent samples. If the Kruskal–Wallis test shows a significant difference between the groups, we used Mann–Whitney *U* test for *post hoc* pairwise comparisons. All *p* values < .05 were considered to be statistically significant. We analyzed the data using the Statistical Package for Social Sciences 21.0 software (IBM-SPSS, Chicago, IL, USA).

Results

A total of 669 participants were included in the study. The mean age was 43 years (range, 1–95), with 139 (20.8%) male and 530 (79.2%) female participants. The main characteristics of study participants are presented in Table 1. The mean and median absolute eosinophil counts of the cohort were 192.6 ± 192.6 cells/μL and 156 cells/μL (range, 0–1,940 cells/μL), respectively, and 23 (3.4%) patients had eosinophilia (eosinophil count ≥ 500 cells/μL).

The mean and median 25(OH)D_3 levels of the patients were 32.2 ± 11.1 ng/mL and 30.9 ng/mL (range, 3.9–70.0 ng/mL), respectively. 25(OH)D_3 status was determined as

being severely deficient in 11 patients (1.6%), deficient in 62 patients (9.3%), insufficient in 238 patients (35.6%), and normal in 358 patients (53.5%).

A comparison of the leukocyte, neutrophil, lymphocyte, and eosinophil counts among the four vitamin D stratification groups is presented in Table 2. The mean/median eosinophil count in the four groups was 267/254 cells/μL, 245/238 cells/μL, 191/159 cells/μL, and 182/146 cells/μL, respectively (p = .001). The difference was significant between the severe deficiency group and each of the other three groups (p = .012, p = .002, and p = .001, respectively). There were also statistical differences between vitamin D deficiency and sufficiency groups (p = .046; Figure 1). There was no statistical difference among the four groups in terms of leukocyte counts (p = .151), neutrophils (p = .177), or lymphocytes (p = .582). There was no sex or age difference between the groups, despite a tendency for older individuals to have a vitamin D deficiency (mean ages of 56 years, 48 years, 45 years, and 43 years, respectively; p = .078).

Discussion

Sun exposure on the skin is the primary source of vitamin D; other sources of vitamin D are foods and supplements. However, a vitamin D deficiency has been reported in healthy individuals in sun-replete areas.[2] This finding may be due to a combination of behavioral factors such as sunscreen use, increased time spent indoors, and clothing coverage.[3] These data support the low levels of $25(OH)D_3$ found in Brazilian patients in our study. Only 53.5% of patients had a $25(OH)D_3$ value within the normal range.

Vitamin D possesses immunomodulatory effects by acting on VDR expressed on B cells, T cells, dendritic cells, and macrophages. Vitamin D has the ability to inhibit both Th1- and Th2-type responses and, in this way, balance the pattern of immune response.[17] Eosinophils expressing VDR and $1,25(OH)_2D_3$ prolong eosinophil survival and upregulate

eosinophil surface expression of CXCR4, an inhibitory chemokine receptor.[18] Vitamin D also reduces eosinophil necrosis and cytolytic release of peroxidase[19] and reduces the production of immunoglobulin E (IgE) and increases expression of interleukin-10, an anti-inflammatory cytokine.[20,21]

Some studies have demonstrated an association between vitamin D and allergic disease: serum 25(OH)D_3 levels were inversely associated with asthma,[4] increased airway responsiveness,[3] the number of exacerbations, asthma severity, and systemic glucocorticoid need.[5–7] Similarly, vitamin D supplementation reduces airway inflammation.[16] Regarding other allergic diseases, 25(OH)D_3-insufficient adult individuals have an increased likelihood of atopic dermatitis[8,9] and allergic rhinitis.[10] 25(OH)D_3 deficiency is not only a marker of allergy but also a predictor of subsequent allergic disease later in life.[12,22]

However, the correlation between 25(OH)D_3 levels and blood eosinophil count is not strongly established. A few studies have noted that lower levels of serum vitamin D are associated with an increased blood eosinophil count,[3,12] but the majority of studies have reported no significant association.[4,5,7,13–16]

The inability of most of these studies to identify this association may be related to the sample tested. All of the studies evaluated populations of allergic or asthmatic patients who may have higher baseline eosinophil levels. In these studies, the mean or median eosinophil count was 236 cells/μL,[4] 254 cells/μL,[7] 311 cells/μL,[15] and 520 cells/μL,[3] and the count ranged from 322 cells/μL to 419 cells/μL[13] and from 390 cells/μL to 590 cells/μL[14] between subgroups. Our study, by contrast, evaluated an unselected population not dominated by people with allergies. We recovered a lower mean/median eosinophil count (193/156 cells/μL) and were able to detect a significant difference in eosinophil levels between the vitamin D stratification groups.

Although serum vitamin D was inversely associated with baseline eosinophil count, it cannot be considered to be associated with eosinophilia. The prevalence of eosinophilia in the sample was low (3.4%), and we were unable to apply a statistical test.

We observed no relation between the number of leukocytes, neutrophils, or lymphocytes among the different groups stratified according to vitamin D level. The inverse correlation of vitamin D with the number of circulating basophils and neutrophils has been reported by only one study. However, despite the statistical significance, the correlation coefficient was very close to zero.[12]

In conclusion, our data suggest that, in an unselected population not dominated by allergic patients, vitamin D deficiency is associated with a higher blood eosinophil count. These results support the possible role of vitamin D in the eosinophil immune response.

Conflicts of interest

The authors have no competing interests.

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Figure 1. Absolute eosinophil count among the four vitamin D stratification groups. Kruskal–Wallis nonparametric test comparing the four groups (p = .001) and Mann–Whitney U test for post hoc pairwise comparisons. Only comparisons with $p \leq .005$ are presented.

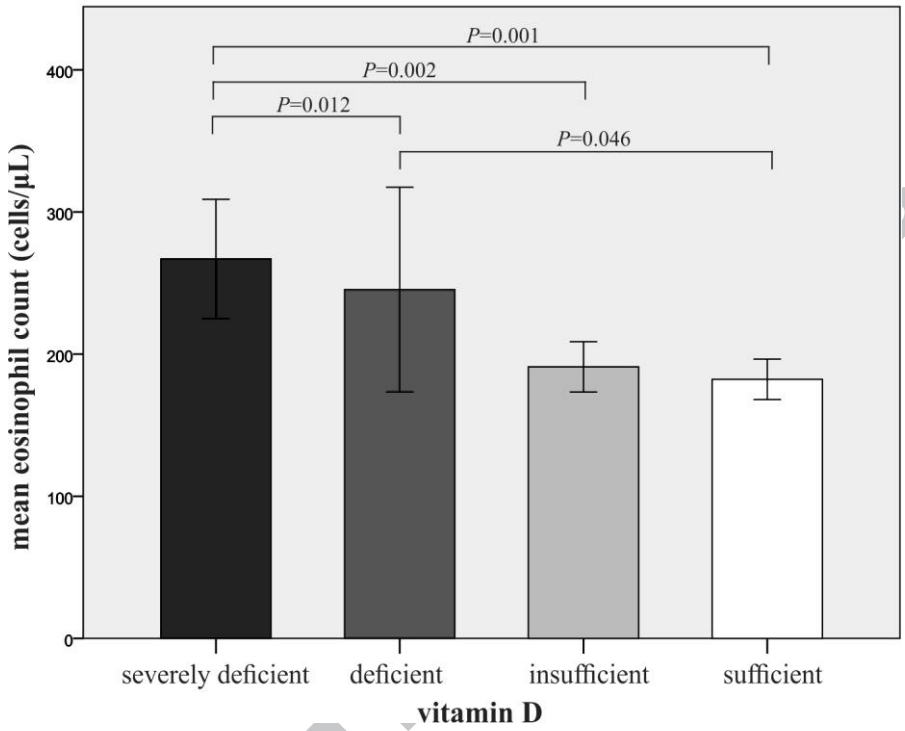

Table 1

Characteristics of Study Participants

Characteristics	N = 669
Sex	
Male, *n* (%)	139 (20.8)
Female, *n* (%)	530 (79.2)
Age, mean ± SD (median)	44.5 ± 19.3 (43)
25(OH)D_3 (ng/mL), mean ± SD (median)	32.2 ± 11.1 (30.9)

Severely deficient (<10 ng/mL), *n* (%)	11 (1.6)
Deficient (≥10 ng/mL and <20 ng/mL), *n* (%)	62 (9.3)
Insufficient (≥20 ng/mL and <30 ng/mL), *n* (%)	238 (35.6)
Sufficient (≥30 ng/mL), *n* (%)	358 (53.5)
Leukocyte count (cells/μL), mean ± SD (median)	6,247.4 ± 1,859.9 (6,000)
Neutrophil count (cells/μL), mean ± SD (median)	3,395.1 ± 1,287.1 (3,233)
Lymphocyte count (cells/μL), mean ± SD (median)	2,190.4 ± 777.3 (2,100)
Eosinophil count (cells/μL), mean ± SD (median)	192.6 ± 192.63 (156)

Note. SD = standard deviation.

Table 2

Comparison of the White Blood Counts among the Vitamin D Deficiency Stratification Groups

	Vitamin D			
	Severely deficient (*n* = 11)	Deficient (*n* = 62)	Insufficient (*n* = 238)	Su
Leukocyte count (cells/μL), mean ± SD (median)	6,309.1 ± 1,676.6 (6,300)	6,548.4 ± 1,683.3 (6,400)	6,037.4 ± 1,621.6 (5,900)	6,3
Neutrophil count (cells/μL), mean ± SD (median)	3,362.5 ± 1,006.2 (3,472)	3,659.7 ± 1,227.4 (3,521)	3,296.3 ± 1,228.9 (3,068)	3,4
Lymphocyte count (cells/μL), mean ± SD (median)	2,185.4 ± 772.7 (2,108)	2,151.7 ± 675.9 (2,115)	2,095.3 ± 577.5 (2,066)	2,2

Eosinophil count (cells/μL), mean ± SD (median)	267.00 ± 62.37 (254)	245.4 ± 283.6 (238)	191.0 ± 138.5 (159)	18

Note. SD = standard deviation.