J Bras Pneumol. 2021;47(1):e20200279
https://dx.doi.org/10.36416/1806-3756/e20200279

Vitamin D: association with eosinophil counts and IgE levels in children with asthma

Claudio Luiz Castro Gomes de Amorim[1,2], Joice Mara de Oliveira[2,3], Antenor Rodrigues[2,4], Karina Couto Furlanetto[2,3], Fabio Pitta[1,2]

1. Programa de Pós-Graduação em Ciências da Saúde, Universidade Estadual de Londrina, Londrina (PR) Brasil.
2. Laboratório de Pesquisa em Fisioterapia Pulmonar, Departamento de Fisioterapia, Universidade Estadual de Londrina, Londrina (PR) Brasil.
3. Centro de Pesquisa e Pós-Graduação, Universidade Pitágoras-Universidade Norte do Paraná – UNOPAR – Londrina (PR) Brasil.
4. Rehabilitation Aimed at Muscle Performance–RAMP–University of Toronto, Toronto, ON, Canada.

Submitted: 3 April 2020.
Accepted: 6 July 2020.

Study carried out at the Ambulatório de Pneumologia Pediátrica, Universidade Estadual de Londrina, Londrina (PR), Brasil.

ABSTRACT

In this cross-sectional study, we investigated the relationship that levels of vitamin D had with eosinophil counts and IgE levels in 26 children with asthma (6-12 years of age) in the city of Londrina, Brazil. Vitamin D levels were found to correlate significantly, albeit moderately, with age (r = −0.51) and eosinophilia (r = −0.49), although not with IgE levels (r = −0.12). When we stratified the sample into two groups by the median vitamin D level (< or ≥ 24 ng/mL), we found that those in the < 24 ng/mL group were older, had higher eosinophil counts, and had higher IgE levels. To our knowledge, this is the first study to show an association between low levels of vitamin D and more pronounced eosinophilia in children with asthma in Brazil.

Keywords: Vitamin D; Asthma; Eosinophils; Eosinophilia; Child.

Inflammation via Th2 lymphocytes is the most common asthma profile in childhood, characterized by the presence of eosinophilia and increased levels of IgE, which are related to the improvement of the disease with the use of corticosteroids.[1-3]

Vitamin D, a liposoluble micronutrient[4] that acts through the vitamin D receptor (VDR),[5] can influence the immunological cascade of asthma by suppressing the response of T2-high lymphocytes and reducing the production of IL-5, thereby decreasing the eosinophil counts and IgE levels.[6] Vitamin D is usually not present in the diet of most people, including that of most Brazilians.[2,7-9] Combined with insufficient sun exposure, that can lead to vitamin D deficiency.

In Brazil, although most people live in regions with adequate sun exposure, vitamin D insufficiency is a common problem that also affects children and is associated with an increased incidence of poorly controlled asthma symptoms.[8,9] In a previous study, involving children with asthma and vitamin D insufficiency, vitamin D supplementation improved asthma control and diminished the risk of exacerbations.[4] Eosinophil counts and IgE levels can also be higher in individuals with vitamin D insufficiency than in those with sufficient levels of the vitamin.[6,10] However, it is questionable whether the reference levels of vitamin D used worldwide (< 20 ng/dL being designated deficient and 20-30 ng/dL being designated insufficient) are applicable as references for all individuals, because the clinical characteristics, place of residence, age, and life habits are not taken into consideration.[9-11]

The relationships that vitamin D levels have with eosinophilia and IgE have not been explored in-depth in children with asthma in Brazil. Despite those affected being subject to clinical specificities and to their own levels of sun exposure, it has been hypothesized that vitamin D levels are associated with those aspects in children in Brazil, as has been observed in populations in other parts of the world. Therefore, the objective of the present exploratory study was to analyze vitamin D levels and their association with eosinophil counts and IgE levels in a sample of schoolchildren with asthma.

The present study was carried out at the Pediatric Pulmonology Outpatient Clinic of Londrina State University, located in the city of Londrina, Brazil. It was a preliminary, exploratory study with an analytical cross-sectional design. The sample was composed of consecutive pediatric patients seen at the outpatient clinic between May and August of 2019 (autumn and winter months), residing in Londrina or the surrounding area. Written informed consent was obtained from the legal guardian of each participant. The project was approved by the human research ethics committee of the institution (Reference no. 3.093.047/2018).

The inclusion criteria were as follows: being 6-12 years of age, being followed at the outpatient clinic, and having received a clinical diagnosis of asthma, in accordance with the GINA criteria[1]; currently using

Correspondence to:
Fabio Pitta. Laboratório de Pesquisa em Fisioterapia Pulmonar (LFIP), Departamento de Fisioterapia, Avenida Robert Koch, 60, Vila Operária, CEP 86038-350, Londrina, PR, Brasil.
Tel.: 55 43 3371-2477. E-mail: fabiopitta@uol.com.br
Financial support: None.

inhaled corticosteroids with no restrictions on the duration of use; being clinically stable, defined as not having needed to use oral corticosteroids to treat an asthma crisis during the last month; not having taken vitamin D supplementation in the previous month; absence of any other pulmonary pathologies, cerebral palsy, gastroesophageal reflux disease, or dysphagia; and having been treated with an antiparasitic agent within the last 12 months. The following exclusion criteria were applied: having experienced an asthma exacerbation that required hospitalization for more than one day or the use of an oral corticosteroid; presenting with comorbidities; using medications that could interfere with the metabolism of vitamin D (e.g., anticonvulsants and systemic antifungal drugs); and no blood sample having been obtained for the quantification of vitamin D.

Patients who met the inclusion criteria, as identified by clinical evaluation, were assessed once. The method of evaluating the serum levels of vitamin D has been described previously,[12,13] reflecting contributions from all sources of this vitamin (i.e., through diet and sun exposure).[14] Previous studies have also described the methods for evaluating serum eosinophils,[3,13] IgE,[15] and the level of asthma control,[1] as well as for diagnosing allergic rhinitis,[16] and performing spirometry.[17,18] The tapering of the inhaled corticosteroid doses and their standardization in budesonide-equivalent doses were as described in the GINA guidelines.[1]

The sample size was determined with a correlation sample size calculator made (http://www.sample-size.net/correlation-sample-size/). Using an alpha of 0.05 and a beta of 0.20, in order to achieve a > 0.60 correlation between the levels of vitamin D and the eosinophils count, we found that the minimum sample required was 19 patients.

In the statistical analysis, the Shapiro-Wilk test was used to analyze the normality of data distribution, the data being expressed as mean and standard deviation or as median and interquartile interval. For analytical purposes, given that the median values measured for vitamin D were not consonant with the reference values typically proposed, the patients were stratified into two groups according to their serum vitamin D levels: those whose level was equal to or above the median for the study sample (24 ng/mL); and those whose level was below that. The median was chosen because, in small samples, it is considered to be more representative. The two groups were compared by using the unpaired Student's t-test or the Mann-Whitney test for continuous variables and the chi-square test for categorical variables. The correlations were evaluated by Spearman's coefficient. The statistical analysis was performed with the IBM SPSS Statistics software package, version 22.0 (IBM Corporation, Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$.

The initial study sample included 27 patients. However, in one case, it was not possible to collect a blood sample for the quantification of the vitamin D level. Therefore, the final sample comprised 26 patients. The baseline characteristics of the patients are described in Table 1. On average, the patients in the sample presented with BMIs within the normal range and vitamin D levels below those considered appropriate, as well as increased IgE levels and eosinophil counts.

Table 1. Characteristics of the patients included in the study (N = 26).[a]

Variable	Result
Age, years	9.5 (7.0-11.0)
Male/female gender, n/n	19/7
BMI, kg/m^2	20 ± 4
Allergic rhinitis	< 100%
Secondhand smoke	< 38%
Uncontrolled asthma	< 35%
Daily dose of inhaled corticosteroid, µg	400 (200-400)
Vitamin D, ng/mL	24 (19-31)
Total IgE, IU/mL	706 (515-1,583)
Eosinophils, %	9.5 ± 6.7
Eosinophils, cells/µL	653 ± 471
FEV_1, % predicted	90 ± 9
Post-BD FEV_1, % predicted	105 ± 14
FEV_1/FVC	78 ± 7
FEV_1/FVC post-BD	84 ± 3
$FEF_{25-75\%}$, % predicted	96 ± 23
Post-BD $FEF_{25-75\%}$, % predicted	136 ± 36

BD: bronchodilator. [a]Values expressed as mean ± SD or median (interquartile interval), except where otherwise indicated.

In comparison with the patients in the ≥ 24 ng/mL vitamin D group, those in the < 24 ng/mL group were older, had higher absolute eosinophil counts, and had higher IgE levels (Table 2). No other significant or borderline statistical differences were observed between the two groups. In the sample as a whole, vitamin D levels showed moderate but statistically significant correlations with age ($r = -0.51$) and with the absolute eosinophil count ($r = -0.49$), although not with the IgE levels ($r = -0.12$; $p = 0.66$). No other significant correlations were observed among the variables studied.

To our knowledge, this is the first study to show that low vitamin D levels are associated with higher absolute eosinophil counts and higher IgE levels in children with asthma in Brazil. However, our results should be interpreted with caution, given that they still do not allow the inference of causality. An association between vitamin D level and age has been previously observed in children and adolescents,[10] although not in children in Brazil. This could be due to lifestyle (such as getting less sun exposure) and to the increased risk of chronic and inflammatory diseases, which increase the metabolism of vitamin D.[8,9]

The role that vitamin D plays at points in the inflammatory cascade in asthma patients is the subject of various ongoing discussions, the outcomes of which

Table 2. Comparison between the groups stratified by the median vitamin D level (24 ng/mL).[a]

Variable	Vitamin D level		p
	< 24 ng/mL (n = 13)	≥ 24 ng/mL (n = 13)	
Age, years	10 (9-11)	8 (7-10)	0.019
Male/female, n/n	9/4	10/3	0.658
BMI, kg/m^2	20 (17-23)	17 (16-23)	0.479
Daily dose of inhaled corticosteroid, μg	400 (200-400)	400 (200-400)	0.880
Uncontrolled asthma	38%	30%	0.999
Secondhand smoke	46%	31%	0.688
Vitamin D, ng/mL	19 ± 4	30 ± 4	< 0.001
Eosinophils, %	11 ± 6	8 ± 7	0.351
Eosinophils, cells/μL	918 ± 464	448 ± 382	0.042
Total IgE, IU/mL	961 (696-2,283)	621 (325-940)	0.046
FVC, % predicted	101 ± 14	108 ± 6	0.548
FEV_1, % predicted	89 ± 11	91 ± 8	0.990
FEF_{25-75}, % predicted	96 ± 23	93 ± 20	0.905

[a]Values expressed as mean ± SD or median (interquartile interval), except where indicated.

have been discrepant. One study of children with asthma (7-14 years of age) in Brazil did not quantify eosinophils but found an inverse association between the levels of IgE and those of vitamin D,[11] whereas another study of children with asthma (6-14 years of age) in Costa Rica showed that vitamin D levels correlated significantly with IgE levels but not with eosinophil counts.[10]

In response to corticosteroids, vitamin D restores the capacity of the T cells to secrete IL-10 (a powerful anti-inflammatory cytokine),[10] thus exerting an immunomodulatory effect[19] and indirectly diminishing the production of IgE,[1,20] given that IgE does not have a VDR and is produced by B lymphocytes. Hypothetically, that would explain why there is not a more robust correlation between vitamin D and IgE, in contrast to what has been observed for eosinophils, which have the VDR[20] and are produced directly by the T2-high lymphocytes. Given that vitamin D can prolong the survival of eosinophils and increase the expression of membrane receptors that inhibit their apoptosis,[20] there is less need to produce new eosinophils in this scenario, which is a possible explanation for the association between vitamin D and eosinophils.

In view of the median vitamin D level found in our sample (24 ng/mL), the internationally accepted cutoff points for vitamin D may not be applicable to children with asthma in Brazil. It is noteworthy that the vitamin D values generally suggested as sufficient, insufficient, and deficient have been based primarily on bone health,[9] involving presumably healthy people,[10] however without considering the specificities related to sun exposure and age.

The limitations of the present study include the relatively small sample size (which might not be representative of the population of children with asthma and precluded multiple regression analysis for the study of independent associations) and the cross-sectional design, which prevented us from establishing causality. Therefore, large, multicenter cohort studies involving nutritional surveys are warranted in order to confirm or refute our findings.

In conclusion, to our knowledge, this is the first study to show an association between vitamin D levels and eosinophil counts in children with asthma in Brazil. However, we were unable to establish a causal relationship between the two. Our findings also suggest that the vitamin D values commonly used are not applicable to children with asthma in Brazil.

AUTHOR CONTRIBUTIONS

CLCGA: study conception and design; data collection; analysis and interpretation of the results; and drafting of the manuscript. JMO, AR, and KCF: analysis and interpretation of the results; and revision of the manuscript. FP: study conception and design; analysis and interpretation of the results; revision of the manuscript; and final approval of the version to be submitted.

REFERENCES

1. Global Initiative for Asthma (GINA) homepage on the Internet]. Bethesda: GINA [cited 2020 Mar 1]. 2019 GINA Report: Global Strategy for Asthma Management and Prevention. Available from: https://ginasthma.org/2019-gina-report-global-strategy-for-asthma-management-and-prevention/
2. Ali NS, Nanji K. A Review on the Role of Vitamin D in Asthma. Cureus. 2017;9(5):e1288. https://doi.org/10.7759/cureus.1288
3. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. J Allergy Clin Immunol. 2010;125(5):995-1000. https://doi.org/10.1016/j.jaci.2010.03.008
4. Martineau AR, Cates CJ, Urashima M, Jensen M, Griffiths AP, Nurmatov U, et al. Vitamin D for the management of asthma. Cochrane Database Syst Rev. 2016;9(9):CD011511. https://doi.

org/10.1002/14651858.CD011511.pub2

5. Canguven O, El Ansari W, Yassin A. Vitamin D Supplementation As a Potential therapeutic Mediator in Asthma: Does Dose Really Matter? a Critical Review of the Literature. Aging Male. 2018;1-8. https://doi.org/10.1080/13685538.2018.1506433
6. Han YY, Forno E, Boutaoui N, Canino G, Celedón JC. Vitamin D insufficiency, TH2 cytokines, and allergy markers in Puerto Rican children with asthma. Ann Allergy Asthma Immunol. 2018;121(4):497-498.e1. https://doi.org/10.1016/j.anai.2018.06.004
7. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr. 2004;80(6 Suppl):1717S-20S. https://doi.org/10.1093/ajcn/80.6.1717S
8. Maeda SS, Borba VZ, Camargo MB, Silva DM, Borges JL, Bandeira F, et al. Recommendations of the Brazilian Society of Endocrinology and Metabology (SBEM) for the diagnosis and treatment of hypovitaminosis D. Arq Bras Endocrinol Metabol. 2014;58(5):411-433. https://doi.org/10.1590/0004-2730000003388
9. Sociedade Brasileira de Pediatria (SBP). Departamento Científico de Endocrinologia [homepage on the Internet]. São Paulo: SBP; c2016 [updated 2016 Dec; cited 2020 Mar 1]. Guia prático de atualização. Hipovitaminose D em pediatria: recomendações para o diagnóstico, tratamento e prevenção. [Adobe Acrobat document, 11p.]. Available from: https://www.sbp.com.br/fileadmin/user_upload/2016/12/Endcrino-Hipovitaminose-D.pdf
10. Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med. 2009;179(9):765-771. https://doi.org/10.1164/rccm.200808-1361OC
11. Santos HLBS, Silva SSE, Paula E, Pereira-Ferrari L, Mikami L, Riedi CA, et al. VITAMIN D RECEPTOR GENE MUTATIONS AND VITAMIN D SERUM LEVELS IN ASTHMATIC CHILDREN. Rev Paul Pediatr. 2018;36(3):269-274. https://doi.org/10.1590/1984-0462/;2018;36;3;00016
12. Määttä AM, Kotaniemi-Syrjänen A, Malmström K, Malmberg LP, Sundvall J, Pelkonen AS, et al. Vitamin D, high-sensitivity C-reactive protein, and airway hyperresponsiveness in infants with recurrent respiratory symptoms. Ann Allergy Asthma Immunol. 2017;119(3):227-231. https://doi.org/10.1016/j.anai.2017.06.014
13. Chinellato I, Piazza M, Sandri M, Paiola G, Tezza G, Boner AL. Correlation between vitamin D serum levels and passive smoking exposure in children with asthma. Allergy Asthma Proc. 2018;39(3):8-14. https://doi.org/10.2500/aap.2018.39.4124
14. Wu AC, Tantisira K, Li L, Fuhlbrigge AL, Weiss ST, Litonjua A, et al. Effect of vitamin D and inhaled corticosteroid treatment on lung function in children. Am J Respir Crit Care Med. 2012;186(6):508-513. https://doi.org/10.1164/rccm.201202-0351OC
15. Kerley CP, Hutchinson K, Cormican L, Faul J, Greally P, Coghlan D, et al. Vitamin D3 for uncontrolled childhood asthma: A pilot study. Pediatr Allergy Immunol. 2016;27(4):404-412. https://doi.org/10.1111/pai.12547
16. Sakano E, Sarinho ESC, Cruz AA, Pastorino AC, Tamashiro E, Kuschnir F, et al. IV Brazilian Consensus on Rhinitis - an update on allergic rhinitis. Braz J Otorhinolaryngol. 2017;S1808-8694(17)30187-8.
17. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26(3):511-522. https://doi.org/10.1183/09031936.05.00035005
18. Pereira CAC. Espirometria. J Pneumol. 2002;28(Suppl 3):S1-S82.
19. Peçanha MB, Freitas RB, Moreira TR, Silva LS, Oliveira LL, Cardoso SA. Prevalence of vitamin D deficiency and its relationship with factors associated with recurrent wheezing. J Bras Pneumol. 2019;45(1):e20170431. https://doi.org/10.1590/1806-3713/e20170431
20. 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. https://doi.org/10.1016/j.hemonc.2017.06.003