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.\(^\text{(1-3)}\)

Vitamin D, a liposoluble micronutrient\(^\text{(4)}\) that acts through the vitamin D receptor (VDR),\(^\text{(5-6)}\) 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.\(^\text{(6)}\)

Vitamin D is usually not present in the diet of most people, including that of most Brazilians.\(^\text{(2,7-9)}\)

Combined with insufficient sun exposure, this 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.\(^\text{(8,10)}\)

In a previous study, involving children with asthma and vitamin D insufficiency, vitamin D supplementation improved asthma control and diminished the risk of exacerbations.\(^\text{(10)}\)

Eosinophil counts and IgE levels can also be higher in individuals with vitamin D insufficiency than in those with sufficient levels of the vitamin.\(^\text{(6,10)}\)

However, it is questionable whether the reference levels of vitamin D used worldwide (\(< 20 \text{ ng/dL being designated deficient and 20-30 ng/dL being designated insufficient}\)) are applicable as references for all individuals, because the clinical characteristics, place of residence, age, and life habits are not taken into consideration.\(^\text{(9-11)}\)

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\(^\text{(10)}\); currently using
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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, reflecting contributions from all sources of this vitamin (i.e., through diet and sun exposure). Previous studies have also described the methods for evaluating serum eosinophils, IgE, and the level of asthma control, as well as for diagnosing allergic rhinitis, and performing spirometry. The tapering of the inhaled corticosteroid doses and their standardization in budesonide-equivalent doses were as described in the GINA guidelines.

The sample size was determined with a correlation sample size calculator made (http://www.sampesize.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.

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, 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.

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
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, 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.

In response to corticosteroids, vitamin D restores the capacity of the T cells to secrete IL-10 (a powerful anti-inflammatory cytokine) thus exerting an immunomodulatory effect and indirectly diminishing the production of IgE, 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 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, 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 commonly used are not applicable to children with asthma in Brazil.

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.

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

| 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/m²                      | 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 |
| FEV₁, % predicted               | 89 ± 11         | 91 ± 8  | 0.990 |
| FEF₂₅₋₇₅, % predicted           | 96 ± 23         | 92 ± 20 | 0.905 |

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