RESEARCH ARTICLE

Vitamin D and asthma in children: insights from total and free 25-Hydroxyvitamin D and vitamin D binding protein measurements

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ABSTRACT

The relationship between serum concentrations of total vitamin D [25(OH)D] and free vitamin D [free 25(OH)D] remains unclear in childhood asthma.

The primary objective was to assess serum levels of 25(OH)D, free 25(OH)D, and vitamin D binding protein (VDBP) in children with asthma compared to healthy children. The secondary objective was to explore potential associations with asthma control and lung function.

50 atopic children, 6-16 years old, with diagnosis of mild-moderate persistent asthma and 40 healthy children of the same age were recruited. In the asthmatic group, the Asthma Control Test (ACT) questionnaire was administered, and lung function was assessed using spirometry. Total 25(OH)D, free 25(OH)D, and VDBP were measured in all participants.

Total 25(OH)D levels were significantly lower in asthmatic children compared to non-asthmatics (mean values: 24.6 ± 8.2 ng/ml *vs*. 29.6 ± 11.7 ng/ml, $p < 0.03$). Free 25(OH)D levels in asthmatic subjects were lower than in non-asthmatic subjects (mean values: 3.2 ± 2 ng/ml *vs*. 6.4 ± 4.5 ng/ml, $p < 0.0001$). The VDBP values were comparable between the two groups (mean values: 239.28 ± 25 µg/ml *vs*. 250 ± 30 µg/ml, $p > 0.05$). There was no significant correlation observed between ACT score, FEV1, and serum levels of vitamin D or VDBP.

Our data show that the total and free serum levels of 25 (OH) D are lower in 32 asthmatic children than healthy ones. We found no correlation between asthma control, lung function and free and total vitamin D levels.

HIGHLIGHTS BOX

What is already known about this topic? Our study confirm that vitamin D deficit is related to asthma. The strength of our study is that it's currently the only study that assessed total and free 25(OH)D all together in children with asthma.

What does this article add to our knowledge? The total and free serum levels of 25 (OH) D are lower in asthmatic children than healthy ones. Despite expectations, in patients who least control asthmatic symptoms and who have worse respiratory function, total and free vitamin D levels are not significantly lower.

How does this study impact current management guidelines? Free vitamin D is rarely investigated in asthmatic children, so further studies are needed to evaluate the relationship about free vitamin D and lung inflammation.

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KEY WORDS

Vitamin D; free vitamin D; VDBP; asthma; asthma control test (ACT); Forced Expiratory Volume in first second (FEV1); children.

INTRODUCTION

Asthma is a chronic disease characterized by airway inflammation. It is defined by a history of wheezing, coughing, dyspnea, and chest tightness that can vary in intensity and over time, accompanied by reversible expiratory airflow limitation (1). Vitamin D plays a crucial role in regulating calcium and phosphate metabolism (2). While its effects on bone health are well-known, recent years have seen growing interest in its unconventional functions (3). Research has unveiled diverse biological actions of vitamin D, including its impact on respiratory health (4) and immunity (5). For skeletal actions, serum concentrations of 25(OH)D ≥30 ng/ml indicate vitamin D sufficiency, whereas concentrations <30 ng/ml are insufficient and levels <20 ng/ml are deficient (6).

Around 85% of serum 25(OH)D is strongly bound to vitamin D binding protein (VDBP), with ~15% loosely bound to albumin and <1% unbound (7). The free hormone hypothesis postulates that only free 25(OH)D concentrations can diffuse across cell membranes, converting to 1.25-dihydroxyvitamin D, which activates the vitamin D receptor (8). Consequently, free 25(OH)D concentrations should be considered better markers of vitamin D status than total serum concentrations (9). This hypothesis also predicts that subjective variability in VDBP levels minimally affects free 25(OH)D concentrations despite influencing total 25(OH)D and the unbound fraction (8). In clinical practice, for example, obese children present free 25(OH)D concentrations similar to those of normal-weight children due to reduced VDBP concentration (10).

In addition to its traditional functions, vitamin D modulates different processes; evidence shows that it has an important role in various allergic diseases (11). Hypovitaminosis D has been associated with an increased risk of asthma development, asthma exacerbations, airway hyperresponsiveness, reduced lung function, and decreased responsiveness to glucocorticoids in both children and adults with asthma (8, 12-15). Low levels of 25(OH) vitamin D have also been found in children with COVID-19 (16).

While some studies show a reduction in asthma exacerbations following vitamin D supplementation, others suggest an increased asthma risk after vitamin D supplementation (17-18). Another study demonstrates that a food supplement containing L. reuteri DSM 17938 (108 CFU) plus vitamin D3 (400 IU) effectively reduces bronchial inflammation (19). The primary endpoint of the study was to evaluate the relationship between serum levels of 25(OH)D3, free 25(OH)D, and VDBP in a population of children with asthma compared to healthy children. In the asthmatic group, the secondary endpoint was to assess whether 25(OH)D and free 25(OH)D serum levels were related to: i) asthma control assessed by ACT; ii) lung function assessed by spirometry; iii) bronchodilation test.

METHODS

Study population

Between October 2018 and March 2019, we selected 50 children (27 male, aged 6-16 years) from patients admitted to the Pediatric Asthma and Respiratory Unit - UOC of Pediatrics, Luigi Vanvitelli University of Campania (**Table 1**).

The inclusion criteria were as follows: (1) Caucasian children; (2) age 6-16; (3) mild-moderate persistent asthma diagnosed by history, clinical findings, and instrumental assessments; (4) House Dust Mite sensitization; (5) good compliance with respiratory function tests.

Exclusion criteria: (1) chronic lung disease or severe asthma; (2) acute infections during recruitment; (3) chronic diseases affecting serum vitamin D levels; (4) vitamin D supplementation.

We included 40 healthy children (23 male) as the control group, excluding obese individuals due to low vitamin D levels and pulmonary function deficit (20).

All participants provided written informed consent, and the Ethics Committee approved the study.

Table 1. *Selected patients: group A asthmatic children; group B non-asthmatic children.*

	Group A asthmatic children	Group B non-asthmatic children
No. PATIENTS	50	40
MALE	27	23
FEMALE	23	17
ATOPY	yes	no

Study design

At the recruitment (T0) all the children were submitted to history and clinical examination. To evaluate their asthma control, the Asthma Control Test (ACT) was performed which consists of five questions, each of which is assigned a score. The scores range from 5 (poor control of asthma) to 25 (complete control of asthma), with higher scores reflecting greater asthma control. An ACT score >19 indicates well-controlled asthma.

Allergic sensitization was assessed by skin prick tests for the main food and environmental allergens according EAACI guidelines. Lung functions were assessed by spirometry (Sensor Medics V max 22) according American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines (21). Lung function parameters including forced expiratory volume in the 1st second (FEV1) and forced vital capacity (FVC) were assessed using spirometry measurements. Percent predicted values for FEV1 and FVC were calculated using the reference equations provided by Quanjer *et al*. (22).

Bronchodilator reversibility test was performed by albuterol 400 µg; a positive test was defined as an increase of FEV1 ≥12% compared to baseline.

In all the patients we took blood samples to measure parathyroid hormone, serum albumin, total 25(OH)D, free 25(OH)D and VDBP.

The levels of total 25(OH)D were measured with the ELISA method by the kit DIAsource ImmunoAssays with an intra and inter-assay coefficient of variation of 5.7%.

Free or unbound concentrations of 25(OH)D in serum were quantified by ELISA with intra and inter-assay coefficient of variation of 5.9%.

Vitamin D binding protein (DBP) was also measured with ELISA method with intra and inter-assay coefficient of variation 12.7%.

Statistical analysis

Descriptive analysis of univariate predictors used categorization of 25(OH)D: sufficient (≥30 µg/L), insufficient (20-29 µg/L), or deficient (<20 µg/L). Mean and standard deviation (SD) were calculated for variables, and groups were compared using Student's t-test. A p value <0.05 indicated statistical significance.

RESULTS

A lower level of 25(OH)D was found in asthmatic individuals compared to non-asthmatics (mean values: 24.6 ± 8.2 ng/ml *vs*. 29.6 ± 11.7 ng/ml, p < 0.03). Similarly, the level of free 25(OH)D in asthmatic subjects was lower than in non-asthmatic subjects (mean values: 3.2 ± 2 ng/ml *vs*. 6.4 ± 4.5 ng/ml, p < 0.0001) (**Figure 1**). The value of VDBP was similar between asthmatic children (mean values: 239.28 ± 25 µg/ml) and non-asthmatic children (250 ± 30 µg/ml VDBP; p = ns) (**Table 2**). None of the 90 children exhibited PTH values outside the normal range (4.6-58.1 pg/ml).

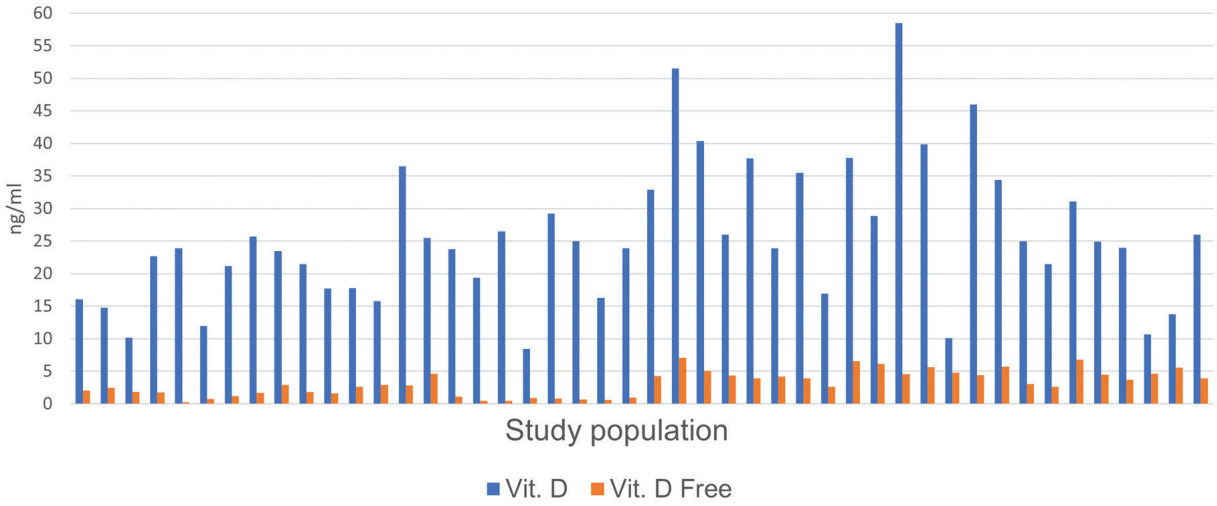

Figure 1. *Total and free vitamin D in the study population.*

Table 2. *Differences of 25(OH)D, free 25(OH)D and value of VDBP in asthmatic individuals compared to non-asthmatics.*

	Group A asthmatic children	Group B non-asthmatic children
MALE/FEMALE	27/23	23/17
TOTAL VIT. D (ng/ml)	24.6 ± 8.2	29.6 ± 11.7
FREE VIT. D	3.2 ± 2	6.4 ± 4.5
VITAMIN D-BINDING PROTEIN (DBP) (microgr/ml)	239.28 ± 25	250 ± 30

Regarding asthma control, no statistically significant differences were found in total 25(OH)D levels (mean values: 25.76 ± 6.2 ng/ml *vs*. 21.82 ± 7.4 ng/ml, p > 0.05) and free 25(OH)D levels (mean values: 3.76 ± 1.9 ng/ml *vs*. 2.8 ± 1.8 ng/ml, p > 0.05) between children with well-controlled asthma (ACT score >19) and those with uncontrolled asthma (ACT score <19). The value of VDBP was similar in both groups (mean values: 235.08 ± 24 µg/ml *vs*. 241.54 ± 19.4 µg/ml, p > 0.05) (**Table 3**, **Figures 2**, **3**).

Table 3. *Differences in total 25(OH)D levels and free 25(OH)D levels between children with well-controlled asthma (ACT score >19) and those with uncontrolled asthma (ACT score <19).*

	ACT = 20-24	ACT < 20
No. PATIENTS	16	34
TOTAL VIT. D (ng/ml)	25.76 ± 6.2	21.82 ± 7.4
FREE VIT. D (ng/ml)	3.76 ± 1.9	2.8 ± 1.8
VITAMIN D-BINDING PROTEIN (DBP) (microgr/ml)	235.08 ± 24	241.54 ± 19.4

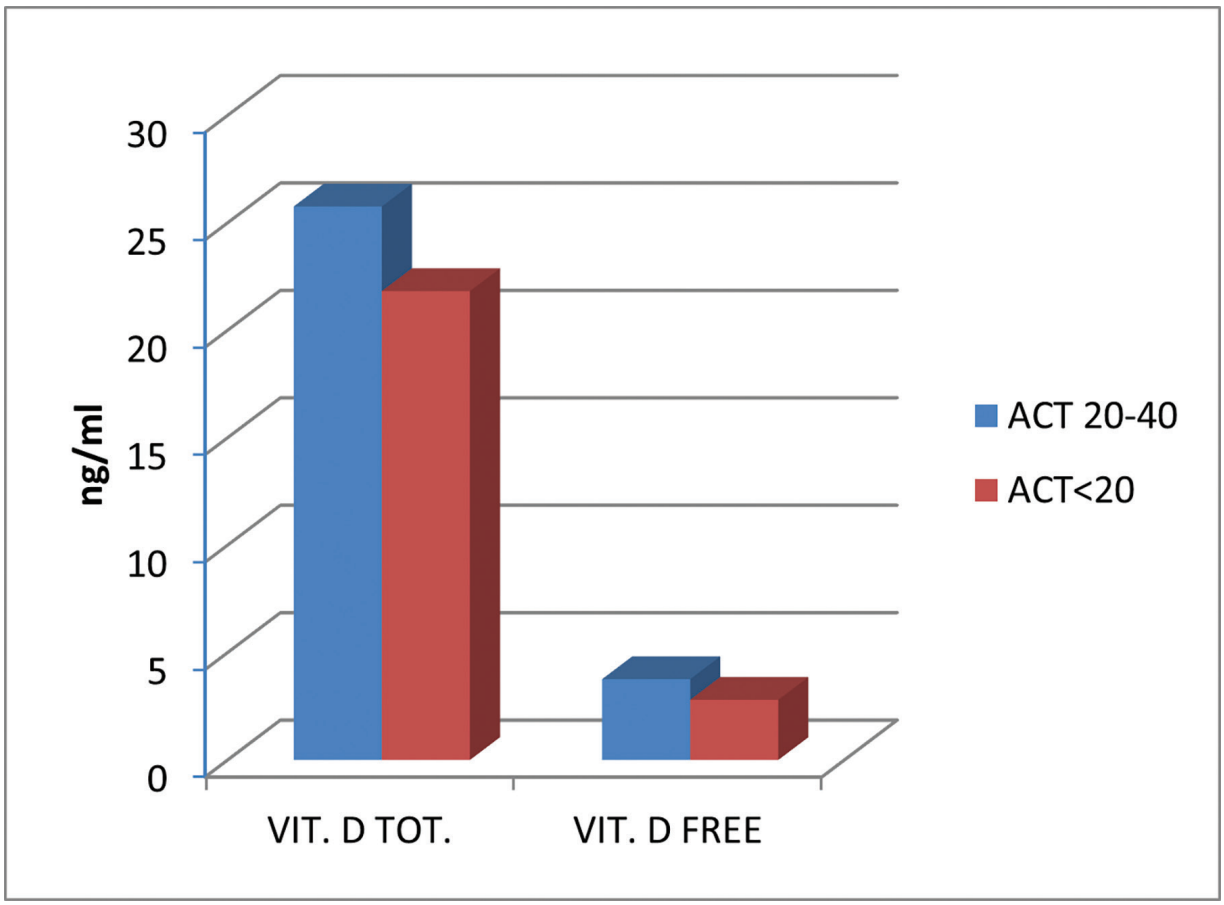

Figure 2. *There are no statistically significant differences between total and free vitamin D levels in patients with partially controlled asthma and patients with uncontrolled asthma (mean values total vitamin D: 25.76 ± 6.2 ng/ml vs. 21.82 ± 7.4 ng/ml, p > 0.05; mean values free vitamin D: 3.76 ± 1.9 ng/ml vs. 2.8 ± 1.8 ng/ml, p > 0.05).*

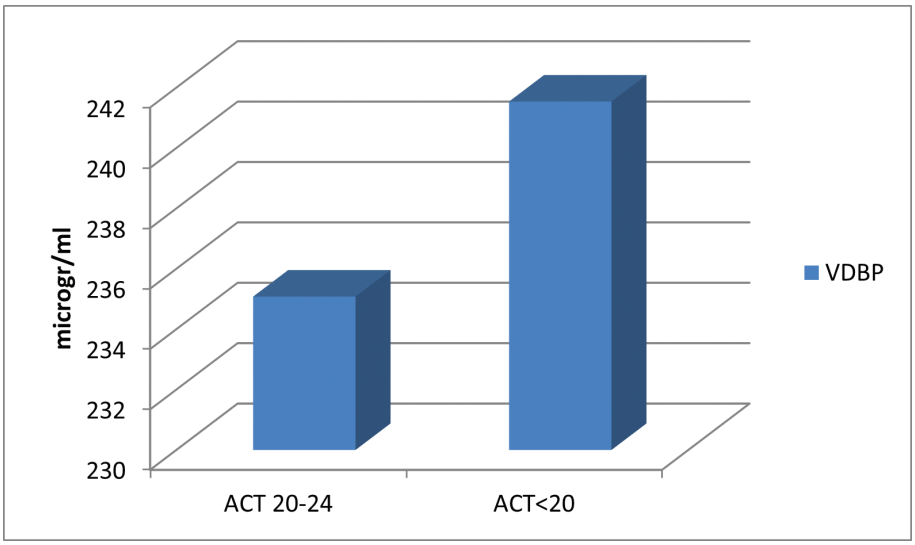

Figure 3. *VDBP values in patients with partially controlled asthma are similar to VDBP values in subjects with uncontrolled asthma (mean values: 235 ± 24 µg/ml vs. 241.54 ± 19.4 µg/ml).*

Asthmatic children were divided into two groups based on a positive or negative bronchodilation test. No significant differences were found in 25(OH)D levels (mean values: 24.8 ± 9.1 ng/ml *vs*. 22.32 ± 4.7 ng/ml, p > 0.05) and free 25(OH)D levels (mean values: 2.98 ± 1.9 ng/ml *vs*. 3.1 ± 2 ng/ml, p > 0.05) between these groups. The value of VDBP showed no statistical difference between the two groups, although patients with a positive bronchodilation test exhibited slightly higher VDBP levels (mean values: 246.6 ± 15 µg/ml *vs*. 239.4 ± 22 µg/ml) (**Table 4**, **Figures 4**, **5**).

Table 4. *Differences of 25(OH)D, free 25(OH)D and value of VDBP in asthmatic children with positive or negative bronchodilation test.*

	ΔFEV1 > 8%	FEV1 < 8%
No. PATIENTS	22	28
TOTAL VIT. D (ng/ml)	24.8 ± 9.1	22.32 ± 4.7
FREE VIT. D (ng/ml)	2.98 ± 1.9	3.1 ± 2
VITAMIN D-BINDING PROTEIN (DBP) (microgr/ml)	246.6 ± 15.8	239.4 ± 22

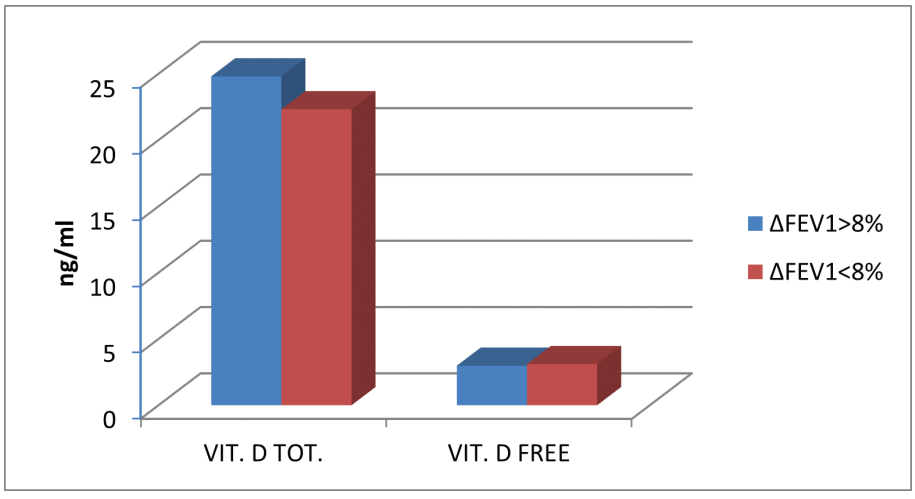

Figure 4. *The values of total and free vitamin D in patients with a positive bronchodilation test are similar to those of patients with a negative bronchodilation test (mean values total vitamin D: 24.8 ± 9.1 ng/ml vs. 22.32 ± 4.7 ng/ml; mean values free vitamin D: 2.98 ± 1.9 ng/ml vs. 3.1 ± 2 ng/ml, p > 0.05).*

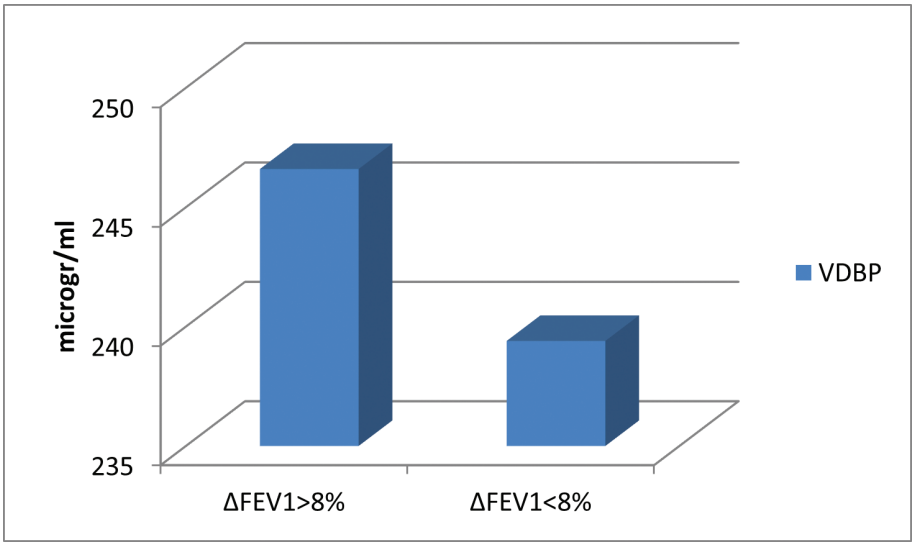

Figure 5. *Children with positive bronchodilatation tend to have higher VDBP values (mean values: 246.6 ± 15.8 µg/ml vs. 239.4 ± 22 µg/ml).*

Concerning lung function, we divided the patients into two groups based on FEV1 levels: children with FEV1 < 80% and children with FEV1 > 80%. The VDBP level was higher but not statistically significant in patients with FEV1 < 80% compared to those with FEV1 > 80% (mean values: 247.36 ± 12.8 µg/ml *vs*. 233.4 ± 28.7 µg/ml). There were no significant differences between the two groups in total 25(OH)D levels (mean values: 25.9 ± 9.4 ng/ml *vs*. 23 ± 7.1 ng/ml, p > 0.05) and free 25(OH)D levels (mean values: 2.96 ± 2.3 ng/ml vs 3.37 ± 1.7 ng/ml, p > 0.05) (**Table 5**).

DISCUSSION

Numerous studies suggest an association between low serum 25(OH)D levels and asthma (23). Our data confirms these findings, with the mean 25(OH)D level significantly lower in the asthmatic group. Recent data underscores that vitamin D metabolism is not well-regulated in asthmatics (24). Powe *et al*. suggested that VDBP gene polymorphisms might affect total 25(OH)D levels, while free 25(OH)D levels seem more related to vitamin D effects (25). Zhao *et al*. proposed that the FokI VDBP gene polymorphism might be linked to pediatric asthma in Caucasian populations (26). Furthermore, our data demonstrates that free 25(OH)D values do not differ from total 25(OH)D values in Caucasian Italian children, yet free vitamin D levels are lower in asthmatics compared to the control group.

Table 5. *Differences of 25(OH)D, free 25(OH)D and value of VDBP in children with FEV1 < 80% and children with FEV1 > 80%.*

	FEV1 < 80%	FEV1 > 80%
No. PATIENTS	18	32
TOTAL VIT. D (ng/ml)	25.9 ± 9.4	23 ± 7.1
FREE VIT. D (ng/ml)	2.96 ± 2.3	3.373 ± 1.7
VITAMIN D-BINDING PROTEIN (DBP) (microgr/ml)	247.36 ± 12.8	233.43 ± 28.7

The majority of vitamin D is carried by VDBP (27). Moreover, VDBP serves several immunomodulatory functions in the lung. Speculation exists that high levels of VDBP in bronchoalveolar lavage fluid (BALF) after allergenic challenges activate alveolar macrophages and induce bronchial inflammation in asthmatics (28). VDBP in BALF might be a marker of airway inflammation (29). Gupta *et al*. discovered an inverse relationship between VDBP levels in BALF and asthma control as assessed by ACT and lung function (30). Additionally, children with severe therapy-resistant asthma (STRA) displayed increased VDBP levels in BALF but not in serum, suggesting upregulation of VDBP exclusively in the lungs. However, the mechanism by which VDBP promotes asthmatic inflammation remains unclear. Chalmers *et al*. observed higher VDBP levels in sputum analysis in bronchiectasis patients with 25(OH)D deficiency (31).

Our study did not find a significant correlation between VDBP levels and lung function, although we were unable

to evaluate VDBP levels in BALF or sputum. In children with a positive bronchodilation test, VDBP serum levels were slightly increased but without statistical significance. Additionally, in assessing asthma control using ACT, children with well-controlled asthma did not show different levels of total and free 25(OH)D compared to those with uncontrolled asthma (32). According to our data, Gergen *et al*. did not find an association between 25(OH)D levels and asthma control assessed by ACT score (33). Conversely, Chinellato *et al*. demonstrated that children with well-controlled asthma had higher serum 25(OH)D levels than children with lower ACT scores (34). There is a lack of consensus among studies on this subject, and moreover, none of these studies evaluated free 25(OH)D serum levels in comparison to total 25(OH)D serum levels.

Lastly, we did not find a correlation between lung function assessed by FEV1 and 25(OH)D levels. Larose *et al*. demonstrated that 25(OH)D serum levels <50 nmol/L were associated with decreased FEV1 in asthmatic adults 35. Another study demonstrated a significant dose-response effect between 25(OH)D serum levels and lung function in children within the community (36).

A limitation of this study is the relatively small number of patients; however, the strength of our study is that it is currently the only study to assess both total and free 25(OH)D levels in children with asthma.

In conclusion, our study confirms that a vitamin D deficiency is related to asthma. Both total and free serum levels of 25(OH)D are lower in asthmatic children compared to healthy ones. Despite expectations, in patients with less controlled asthma symptoms and worse respiratory function, total and free vitamin D levels are not significantly lower. Free vitamin D is rarely investigated in this type of patients; therefore, further studies are needed to evaluate the relationship between free vitamin D and lung inflammation.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

Conceptualization: AA and LR; methodology: AA, GRU and LR; investigation: AA, CI and LR; data curation: AA, GRU; writing-original draft preparation: AA; writing-review and editing: AA, GD, CC, MDG, PV, GP. All the Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

The request to the Ethics Committee for carrying out the study is protocol 266/2016.

Animal studies

N/A.

Data sharing and data accessibility

Data are available upon motivated request to Corresponding Author.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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