 DOI: 10.62838/amsm-2026-0028

RESEARCH ARTICLE

Evaluation of serum vitamin D_3 role in relation to asthma severity, control status, and eosinophilic phenotype: evidence from an Iraqi cohort within the global initiative for asthma 2025 guideline

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Abstract

Background: Vitamin D_3 is involved in immunological actions, and recent data indicate its role in decreasing airway inflammation, which is one of the cornerstones of asthma.

Objectives: To evaluate serum vitamin D_3 levels in Iraqi asthmatic patients compared to healthy controls, and to investigate their relationships with asthma severity, Global Initiative for Asthma (GINA) 2025-defined control status, and the eosinophilic phenotype.

Methods: A case-control study of 90 asthmatics and 90 healthy subjects was performed. Asthma severity was classified as moderate (n=56) or severe (n=34); control status was categorized as controlled (n=31), partially controlled (n=34), or uncontrolled (n=25), based on GINA 2025 guidelines. Serum vitamin D_3 levels were measured by AFIAS-6 immunoanalyzer using fluorescence immunoassay. Statistical analyses were conducted by Mann-Whitney U test, Kruskal-Wallis's test, Spearman correlation, and ROC curve analysis.

Results: Vitamin D_3 levels were significantly lower in asthma patients compared to healthy controls ($p<0.001$), and were markedly lower in severe asthma compared to moderate asthma ($p<0.0001$). Additionally, a significant progressive decrease in its levels was observed with worsening disease control ($p=0.0006$), along with a significant positive correlation between vitamin D_3 and ACT score ($p<0.001$).

Conclusion: Vitamin D_3 insufficiency is substantially related to greater severity of asthma and poor asthma control among Iraqi patients; therefore, it could serve as a potential biomarker and a target for treatment.

Keywords: vitamin D_3, asthma severity, Eosinophilic Phenotype, GINA classification, Iraqi patients

Received 7 june 2026 / Accepted 31 august 2026

Introduction

Asthma is a chronic heterogeneous inflammatory disease of the airways that affects an estimated 300 million people worldwide [1] and is associated with significant morbidity and health-care burden. The pathophysiology is characterized by a complex interplay of genetic predisposition, environmental exposures, and dysregulated immune responses, primarily T-helper 2 (Th2)–driven inflammation in the vast majority of phenotypes [2]. The significance of classification of asthma not only based on severity but also on control has been emphasized by recent international recommendations, including the Global Initiative for Asthma (GINA) 2025 guidelines, where the control status is recognized to better reflect day-to-day disease burden and sets therapeutic direction [3]. Vitamin D_3 (25-hydroxyvitamin D) has become a pivotal immunomodulatory hormone with pleiotropic roles beyond calcium homeostasis and bone metabolism [4]. As VDR is present on various immune cells such as T lymphocytes, B lymphocytes, dendritic cells, and macrophages, it may directly affect inflammatory responses [5]. In asthma, vitamin D_3 protects against the development of asthma like phenotypes through multiple mechanisms, including suppression of Th2 cytokine production (particularly IL-13 and IL-4), stimulation of Treg differentiation, increased expression of the glucocorticoid receptor and steroid responsiveness, and preservation of airway epithelial barrier integrity [6, 7]. Epidemiological studies have shown consistently lower vitamin D status in asthmatic cohorts as compared to healthy control populations, and argued for an association of deficiency with increased risk of exacerbations, impaired lung function, and poor symptom control [8, 9]. However, clear heterogeneity between studies was noted in the magnitude of the associations between immunopathologies, which may be driven by differences in population demographics, geographic latitude, sun exposure, dietary-related factors, and polymorphisms in vitamin D metabolic pathways [10]. The Persian Gulf region, including Iraq, is particularly relevant because of the often-high ambient sunlight associated with high traffic area where penetration solar radiation is negative, coupled with paradoxically highly prevalent vitamin D deficiency arising in part from cultural, clothing practices and the preferences

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for staying indoors and the relatively little fortification of food [11]. Limited evidence is available on vitamin D associations with both disease severity (moderate vs. severe) and multidimensional control status (controlled, partially controlled, uncontrolled) defined by current GINA criteria in Iraqi patients, although the importance of vitamin D in asthma management is appreciated [12, 13]. Therefore, the present study aimed to: evaluate the serum vitamin D_3 levels between asthmatic patients and healthy controls in an Iraqi cohort; examine associations between vitamin D_3 and asthma severity (moderate vs. severe) and assess its level differences across control categories (controlled, partially controlled, uncontrolled) according to GINA classification.

Methods

Study Design and Population

A case-control study was conducted at Al-Imamain Al-Kadhimain Medical City, and in the laboratories of the Department of Chemistry and Biochemistry at the College of Medicine, University of Al-Nahrain in Baghdad, from April 1, 2025, to December 1, 2025. Participants were recruited using a simple random sampling method during one month. The study included 180 participants, comprising 90 adults with clinically diagnosed asthma and 90 healthy controls without respiratory disease. Asthma patients were diagnosed and classified under the supervision of a specialist physician according to GINA 2025 guidelines [3]. Based on these guidelines, asthma severity is assessed retrospectively depending on the level of treatment required to achieve symptom control. The current study cohort comprised patients classified into two severity categories: moderate and severe asthma. Patients with mild asthma were not included in this study due to recruitment challenges in our clinical setting. Moreover, based on symptom control and the response to treatment, patients were divided into three groups: controlled, partially controlled, and uncontrolled.

Sample Size Calculation

An a priori sample size calculation was conducted utilizing G*Power software (version 3.1.9.4). Based on a two-tailed Wilcoxon–Mann–Whitney test with a medium effect size (Cohen's d = 0.50), an alpha level of 0.05, and a statistical power of 80%, a minimum of 67 participants per group (total N = 134) was determined to be necessary. To enhance the robustness of the findings and safeguard against potential data loss, the final cohort was expanded to 90 asthmatic patients and 90 healthy controls, resulting in a total sample size of 180 participants.

Inclusion and Exclusion Criteria:

Participants were adults aged 18–70 years with a physician-confirmed diagnosis of asthma. Exclusion criteria included: age <18 years; mild asthma; other chronic respiratory diseases (e.g., chronic obstructive pulmonary disease or pulmonary fibrosis); pregnancy or lactating women; a history of advanced liver disease (e.g., cirrhosis, advanced fibrosis, cholestatic liver disease, or end-stage liver disease) or chronic kidney disease; advanced malignant tumors; and active smoking. Healthy controls had no history of asthma, atopic diseases, or chronic respiratory conditions.

Anthropometric Measurements

The body mass index (BMI) was calculated using weight in kilograms divided by height in meters squared. Obesity was defined as a (BMI ≥30 kg/m²). The Arabic validated version of the Asthma Control Test (ACT) was used to assess asthma control test. The ACT is a self-administered questionnaire, consisting of 5-items assessing symptoms and reliever use in the previous 4 weeks. Scores can range from a total of 5 at the low end (very poor control) to 25 at the high end (excellent control) [14].

Ethical Considerations

Approval for the study protocol by the IRB (Institutional Review Board) of the College of Medicine, University of Al-Nahrain was granted on 11-02-2025 with the decision number: 20241022. All procedures were performed in accordance with the principles of the Declaration of Helsinki (1964) and its amendments. All participants provided oral informed consent before enrolment.

Laboratory Measurements

All blood samples for both asthmatic patients and healthy controls were collected within the same one-month period to minimize potential seasonal variation in serum vitamin D levels. Five milliliters of venous blood were collected under sterile conditions. Out of this, 2 mL was transferred into an EDTA tube for eosinophil count, while the remaining blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for ten minutes. The serum was isolated and stored at -80°C until analysis. Vitamin D_3 (25-hydroxyvitamin D) concentrations were quantitatively measured using the AFIAS-6 immunoanalyzer (Boditech Med Inc., Chuncheon, Republic of Korea), which operates on the principle of fluorescence immunoassay. The AFIAS-6 system utilizes a ready-to-use cartridge containing fluorescence-labeled detection antibodies specific for 25-hydroxyvitamin D. The assay was performed according to the manufacturer's instructions. The assay's analytical sensitivity was 2.0 ng/mL, and the test displayed linearity throughout a range of 4-120 ng/mL. Vitamin D_3 status was categorized as deficient (<20 ng/mL), insufficient (20-29 ng/mL), or sufficient (≥30 ng/mL). Complete blood count with differential was performed using an automated hematology analyzer (Sysmex XN-1000, Kobe, Japan) to determine peripheral eosinophil counts. Eosinophilic asthma was defined as an eosinophil count ≥0.4 × 10^3/μL [15–17].

Statistical Analysis

Statistical analyses were performed using JASP (version 0.16.4, JASP Team, Amsterdam, Netherlands) and GraphPad Prism (version 9.5, GraphPad Software, San Diego, CA, USA). Normality of data distribution was assessed using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or median with interquartile range (Q1-Q3) for non-normally distributed data. Group comparisons were performed using: Unpaired t-test with Welch's correction for two-group comparisons of normally distributed data; Mann-Whitney U test for two-group comparisons of non-normally distributed data; and Kruskal-Wallis test followed by Dunn's post-hoc analysis for three-group comparisons. Categorical variables were compared using the Chi-square test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of vitamin D_3 in discriminating asthmatic patients from healthy controls, calculating area under the curve (AUC), optimal cut-off value (Youden index), sensitivity, and specificity. Statistical significance was set at two-tailed P<0.05.

Results

Demographic and Clinical Characteristics

A total of 180 subjects (90 asthma patients; 90 healthy controls) were included in this case-control study. Of the patients, 55 (61.1%) were women, and 35 (38.9%) were men; the sex difference between groups was not significant (χ^2=0.023, p=0.879). Patients had a mean age of 46.00±14.87 years, with a corresponding mean age of 48.30±12.69 years for controls (p=0.265). There was no significant difference in BMI values (30.16±5.55 vs. 29.36±3.43 kg/m²; p=0.242). Based on GINA severity classification, 56 patients (62.2%) were found to have moderate asthma, and 34 (37.8%) had severe asthma. According to GINA 2025 control criteria, 31 (34.4%) patients were classified as controlled, 34 (37.8%) as partially controlled, and 25 (27.8%) as uncontrolled. ACT scores identified that 41.1% (37) of study patients had well-controlled asthma (ACT ≥19) and 58.9% (53) had poor control (ACT <19).

Table I. Comparison of Serum Vitamin D_3 Levels between Asthmatic Patients and Healthy Controls

Parameter	Patient (n=90)	Control (n=90)	p-value
Median (Q1-Q3), ng/mL	19.74 (14.1-23.9)	26.01 (20.5-32.2)	< 0.001
Mean ± SD, ng/mL	19.29 ± 6.369	27.13 ± 9.322	

Legend: Statistical comparisons between groups were performed using the Mann–Whitney U test based on data distributions and central tendencies, summarized as Median (Q1–Q3). Mean values are presented for descriptive purposes only. (Q1–Q3) represent the 25th and 75th percentiles

Vitamin D_3 in Asthmatic Patients and Healthy Controls

Serum vitamin D_3 levels were found to be significantly reduced in asthmatic patients compared to the healthy controls Table I. The median vitamin D_3 levels within the asthmatic subjects were 19.74 ng/mL [IQR: (14.1-23.9)] for comparisons with controls, which had 26.01 ng/mL [IQR: (20.5-32.2)] and corresponded to a 24.1% reduction (P < 0.001). Using the definitions of sufficiency, insufficiency, and deficiency, 53.3% of the asthmatic patients were classified as vitamin D deficient (<20 ng/mL), 42.2% as insufficient (20-29 ng/mL), and only 4.4% as sufficient (≥30 ng/mL). In healthy controls, by contrast, 24.4% were deficient, 41.1% insufficient, and 34.4% sufficient.

Associations of Vitamin D_3 with Severity of Asthmatic Patients

As shown in Table II; Figure 1. Comparative analysis of vitamin D_3 levels between moderate (n=56) and severe (n=34) asthma revealed a significant difference between the two groups. Vitamin D_3 was lower in severe as compared to moderate asthma, with a median of 14.6 ng/mL [IQR: (11.6 – 19.3)] vs. 22.2 ng/mL [IQR: (17.6 – 25.3)] (P < 0.0001).

Table II. Comparison of Serum Vitamin D_3 Levels between Moderate and Severe Asthma

Parameter	Severe (n=34)	Moderate (n=56)	p-value
Median (Q1-Q3), ng/mL	14.6 (11.6 – 19.3)	22.2 (17.6 – 25.3)	<0.0001
Mean ± SD, ng/mL	15.3 ± 5.2	21.7 ± 5.8	

Legend: Statistical comparisons between groups were performed using the Mann–Whitney U test based on data distributions and central tendencies, summarized as Median (Q1–Q3). Mean values are presented for descriptive purposes only. (Q1–Q3) represent the 25th and 75th percentiles.

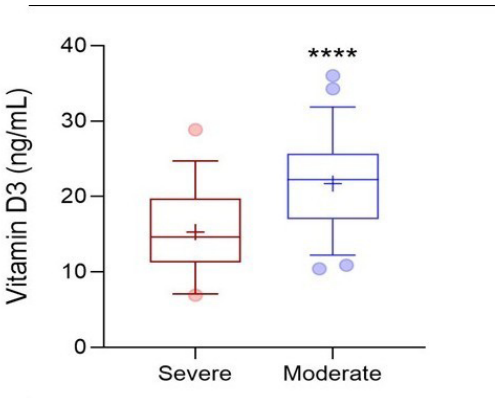

Figure 1. Box-plot comparison of serum Vitamin D_3 levels between moderate and severe asthma. Boxes depict IQR (25th-75th percentiles); horizontal lines indicate medians; whiskers extend to non-outlier minima and maxima; circles denote outliers. Severe asthma shows significantly lower Vitamin D_3 compared to moderate asthma (p< 0.0001).

Associations of vitamin D_3 with asthma control status

Kruskal-Wallis Test across control categories as defined by GINA confirmed a significant, step-wise association between deteriorating asthma control and vitamin D_3 levels Table III; Figure 2. Differences among groups were highly significant according to this test (P= 0.0006). Results: Vitamin D_3 concentrations were highest in the controlled group [median: 22.4 ng/mL, IQR: (18.1–24.5)] and lower in partially controlled [median: 19.8 ng/mL, IQR:(15.9–24.4)] and lowest in the uncontrolled group [median: 14.8

ng/mL, IQR: (13.1–18.0)]. Post hoc analysis showed two important comparisons—controlled > uncontrolled and partially controlled > uncontrolled; controlled and partially controlled were not significantly different.

Table III. Comparison of Serum Vitamin D3 Levels Across Asthma Control Categories

Parameter	Controlled (n=31)	Partially Controlled (n=34)	Uncontrolled (n=25)	p-value	Post-hoc analysis
Median (Q1-Q3)	22.4 (18.1 – 24.5)	19.8 (15.9 – 24.4)	14.8 (13.1 – 18.0)	0.0006	C > U
Mean ± SD	21.8 ± 6.60	19.6 ± 6.29	15.6 ± 4.40		P > U
					C = P

Legend: Statistical comparisons among the three asthma control groups (Controlled (C), Partially Controlled (P), Uncontrolled (U)) were performed using the Kruskal–Wallis test based on data distributions and central tendencies, summarized as Median (Q1–Q3), followed by Dunn's post-hoc analysis for pairwise comparisons. Mean ± SD values are presented for descriptive purposes only and are not implied in the P-value calculations. The symbols in the "Post-hoc analysis" column indicate the direction of significant differences between groups.

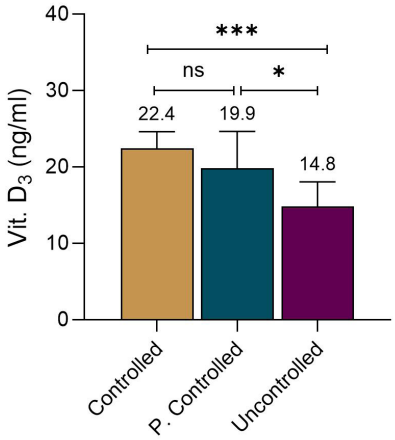

Figure 2. Bar chart illustrating median serum Vitamin D3 concentrations with IQR error bars in asthmatic patients classified by control status according to the GINA guideline. Significant differences: Uncontrolled < Controlled; Uncontrolled < Partially Controlled (P=0.0006). ns = not significant. P. Controlled = Partially Controlled.

Subgroup Analysis

Eosinophilic phenotype was significantly inversely associated with vitamin D_3 Table IV. In those with eosinophilic asthma (n=55; eosinophils ≥0.4 × 10^3/**µ**L), vitamin D_3 levels were significantly lower [median: 17.8 ng/mL, IQR: (13.8-22.1)] than in non-eosinophilic patients [median: 22.2 ng/mL, IQR: 17.9-24.8); P=0.003]. Additional validation of these results stratified by ACT score demonstrated patients with poorly controlled asthma (ACT <19, n=53) had significantly lower levels of vitamin D_3 [(median: 16.8 ng/mL, IQR: (13.2-20.9)] compared to well-controlled patients (ACT ≥19, n=37) [median: 22.4 ng/mL, IQR: (18.1-26.0); P<0.001]. Slightly lower levels in obese [BMI ≥30, n=45; median: 18.6 ng/mL, IQR: (13.2-22.4)] than non-obese [BMI <30, n=45; median: 20.4 ng/mL, IQR: (15.8-24.4)] asthmatic patients (P= 0.154) were observed, but did not reach significant difference, although agreed with known inverse relationships between adiposity and vitamin D bioavailability. No statistically significant difference in serum vitamin D levels was observed between patients receiving inhaled corticosteroids (ICS) or combined ICS/LABA therapy and those not receiving these treatments (p > 0.05 for all comparisons).

Table IV. Subgroup Analysis of Vitamin D3 According to Eosinophilic Status, Asthma Control Test Score and Obese vs. Non-Obese

Subgroup	Category	n	median (Q1-Q3)	p-value
Eosinophilic status	Eosinophilic (≥0.4 × 10^3/µL)	55	17.8 (13.8-22.1)	0.003
	Non-eosinophilic (<0.4 × 10^3/µL)	35	22.2 (17.9-24.8)	
ACT score	Well-controlled (ACT ≥19)	37	22.4 (18.1-26.0)	<0.001
	Poorly controlled (ACT <19)	53	16.8 (13.2-20.9)	
Obesity	Obese (BMI ≥30)	45	18.6 (13.2-22.4)	0.154
	Non-obese (BMI <30)	45	20.4 (15.8-24.4)	
Inhaled Corticosteroid (ICS)	Yes	10	20.3 (15.9-24.1)	0.074
	No	80	19.1 (14.1-23.9)	
ICS / LABA Combination	Yes	68	20.4 (14.6-24.4)	0.714
	No	22	16.5 (13.1-20.9)	

Legend: Statistical comparisons between groups were performed using the Mann–Whitney U test based on data distributions and central tendencies, summarized as Median (Q1–Q3).

Correlation Analysis

Vitamin D_3 levels showed a moderate positive correlation with ACT score (r = 0.455, p < 0.001) and a moderate negative correlation with eosinophil count (r = –0.395, p < 0.001), suggesting a potential link between vitamin D status, asthma control, and eosinophilic inflammation.

Diagnostic Performance of Vitamin D_3

Discriminative ability of serum vitamin D_3 to distinguish asthmatic patients from healthy controls was assessed using ROC curve analysis. Vitamin D_3 showed moderate diagnostic performance [AUC 0.751 (95% CI: 0.682-0.813; P<0.0001)]. The optimal cut-off value (≤25.1 ng/mL) yielded high sensitivity of 83.3% but lower specificity of 55.5%. This means that a lower vitamin D_3 level is frequent in patients with asthma, but also in healthy people due to environmental and lifestyle features. Thus, low vitamin D_3, while supporting the identification of asthma, in isolation is not effective as a mono-diagnostic marker and serves best as a supportive potential marker to be used within a multi-analyte panel.

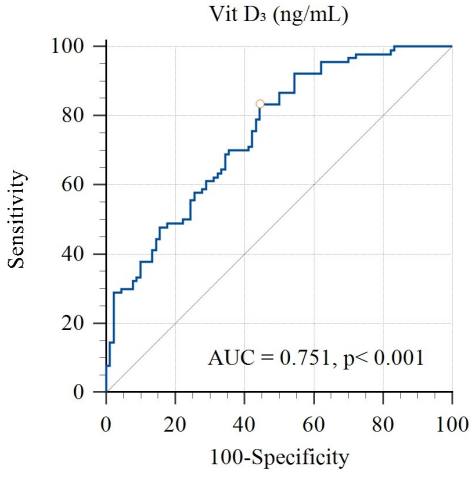

Figure 3. Receiver Operating Characteristic (ROC) Curve for (Vit. D_3) in Classifying Patients from Controls.

Discussion

The current study found that serum vitamin D_3 levels were noticeably lower in asthmatic patients than healthy controls (P<0.001). This principal finding conforms to modern international literature, which features an international prevalence of low antioxidant vitamins and pro-hormone vitamins in chronic inflammatory lung ailments [18]. The dynamic holds particularly true in the case of the Middle East, including Iraq. However, with abundant ambient sunlight, a recent epidemiological review has shown that a large proportion of the population has hidden vitamin D deficiency due to elements such as arid climates limiting outdoor activity, traditional dress that inhibits skin synthesis, and lack of food fortification [19]. Our findings suggest that the baseline regional deficiency is associated with further depletion in asthmatic patients. Analysis stratifying the cohort by disease severity and control showed a significant reduction in vitamin D_3 levels in our cohort of patients with severe asthma (p<0.0001) vs. moderate asthma. Notably, Vitamin D_3 levels showed stepwise reductions across

GINA-defined control categories, with the lowest levels observed in patients with uncontrolled asthma (p=0.0006). This pattern is consistent with prior clinical observations linking lower vitamin D levels with increased asthma severity and exacerbation risk [20]. To further elucidate the immunomodulatory role of vitamin D_3, we evaluated its relationship with specific inflammatory phenotypes and airway remodeling biomarkers. We observed a significant inverse association between vitamin D_3 levels and the eosinophilic asthma phenotype (p=0.003). Recent multi-center data (2025) confirm that low serum 25-hydroxyvitamin D levels independently increase the odds of developing eosinophilic asthma [21]. Vitamin D_3 has been reported to play an immunomodulatory role in regulating inflammatory pathways, including Th2 responses [22, 23]. In the present study, lower vitamin D_3 levels were associated with increased eosinophilic inflammation and more severe disease phenotypes. However, this relationship should be interpreted with caution, as causality cannot be established within the framework of a case-control design.

Interestingly, while obesity is widely recognized as a factor that can sequester vitamin D and reduce its bioavailability, our subgroup analysis did not yield a statistically significant difference in vitamin D_3 levels between obese and non-obese asthmatic patients (p=0.154). This non-significant finding suggests that the profound vitamin D depletion in this cohort may be more closely related to the underlying asthma pathophysiology and the shared environmental/lifestyle factors prevalent in Iraq [19], rather than being independently dictated by adiposity. The use of inhaled corticosteroids (ICS), either alone or in combination with long-acting β_2-agonists (LABA), did not show a statistically significant effect on serum vitamin D levels in this study. This suggests that the observed association between vitamin D levels and asthma severity is unlikely to be substantially confounded by standard inhaled therapy. Finally, the diagnostic performance of vitamin D_3 was evaluated using ROC curve analysis, which indicated a potential capacity to discriminate asthmatic patients from healthy individuals (p<0.0001). However, because vitamin D deficiency is highly prevalent in the general population due to aforementioned lifestyle factors [19], isolated vitamin D_3 levels may not be suitable as a standalone diagnostic tool.

Study's Limitations

Importantly, the case-control and cross-sectional nature of this study limits the ability to establish causal relationships between vitamin D_3 levels and asthma-related outcomes. The observed associations should therefore be interpreted with caution. Reverse causation remains a plausible explanation, as patients with more severe or poorly controlled asthma may have reduced outdoor activity and sun exposure, leading to lower vitamin D_3 levels. Additionally, residual confounding cannot be excluded. Future longitudinal and interventional studies are warranted to clarify the directionality of these associations.

Furthermore, while our findings provide valuable insights into the Iraqi population, the single-center nature of this study and its specific geographical context mean that these results may not be fully generalizable to other populations with different environmental exposures and baseline vitamin D statuses. Larger, multi-center longitudinal studies are required to confirm these associations.

Conclusions

Vitamin D_3 levels are significantly reduced in asthmatic patients. Deficiency is associated with increased severity and poor control in adult patients, with a potential role in modulating eosinophilic airway inflammation. These findings support the consideration that vitamin D_3 may be a useful prognostic indicator and a potential adjunctive target in asthma management; however, it should not be used as a standalone diagnostic marker.

Authors' contributions

H.Y.A (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing)

H.A.A (Conceptualization; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing)

R.J.M (Conceptualization; Investigation; Methodology; Supervision; Writing – review & editing)

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

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