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The interplay between serum Interleukin-33 and vitamin D3 in childhood asthma: Diagnostic utility and severity predictors in an Iraqi community

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ABSTRACT

Background: The epithelial alarmin Interleukin-33 (IL-33) and the immunomodulator vitamin D3 are implicated in asthma pathogenesis, yet their combined clinical utility and interplay in pediatric populations remain underexplored.

Objectives: This study aimed to investigate the diagnostic performance and predictive value of serum IL-33 and vitamin D3 in childhood asthma, and their association with disease severity.

Materials and methods: A case-control study was conducted involving 120 children (60 asthmatics and 60 age- and gender-matched healthy controls) in Karbala, Iraq. Serum levels of IL-33 and 25-hydroxyvitamin D3 were quantified using enzyme-linked immunosorbent assay (ELISA). Diagnostic utility was evaluated via Receiver Operating Characteristic (ROC) curve analysis, and independent predictors were identified using multivariate binary logistic regression.

Results: Asthmatic children exhibited significantly elevated IL-33 (395.56±218.75 vs 159.95±55.32 ng/L, p<0.001) and lower vitamin D3 (17.01±7.38 vs 28.20±10.60 ng/mL, p<0.001) than controls. A strong inverse correlation was observed between IL-33 and Vitamin D3 (r = -0.614, p<0.001), both of which showed a dose-dependent relationship with asthma severity. ROC analysis revealed exceptional diagnostic accuracy for IL-33 (AUC=0.933). Multivariate regression identified body mass index (aOR = 1.528, p=0.014) and IL-33 (aOR=1.015 per 1 ng/L increase, p=0.020) as significant independent predictors of asthma, yielding an overall model accuracy of 95.8%. Notably, male asthmatics exhibited significantly lower vitamin D3 levels than females (p<0.001).

Conclusion: The inverse relationship between elevated IL-33 and vitamin D3 deficiency underscores their synergistic role in asthma exacerbation. IL-33 serves as a highly accurate diagnostic biomarker, highlighting the potential for targeted anti-alarmin therapies and gender-stratified nutritional interventions in pediatric asthma management.

Introduction

Asthma is one of the most prevalent chronic respiratory diseases globally, imposing a substantial burden on healthcare systems and diminishing the quality of life for millions of individuals. According to the Global Burden of Disease (GBD) study and the World Health Organization (WHO), asthma affected an estimated 262 million people worldwide in 2019 and accounted for approximately 455,000 deaths.[1] The

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prevalence of childhood asthma has been rising steadily, particularly in developing nations and the Middle East, driven by a complex interplay of genetic predisposition, urbanization, and environmental exposures such as passive smoking.[2] In Iraq, epidemiological studies have reported childhood asthma prevalence rates ranging from 7% to over 22% in certain urban centers, highlighting a critical regional health challenge.[3] Despite advancements in therapeutic strategies, a significant proportion of pediatric patients continue to experience recurrent exacerbations and suboptimal disease control, underscoring the urgent need to elucidate the precise molecular and immunological mechanisms driving asthma pathogenesis.

Recent immunological research has increasingly focused on the role of airway epithelial cells, not merely as a physical barrier, but as frontline regulators of innate immunity. Upon exposure to environmental triggers such as allergens, pollutants, or cold air, damaged epithelial cells rapidly release a group of cytokines known as "alarmins," which include Thymic Stromal Lymphopoietin (TSLP), Interleukin-25 (IL-25), and Interleukin-33 (IL-33).[4] Among these, IL-33, a member of the IL-1 cytokine superfamily discovered by Schmitz et al. in 2005, has emerged as a crucial mediator of type-2 airway inflammation.[5] IL-33 exerts its biological effects by binding to its specific receptor, ST2 (IL-1RL1), which is widely expressed on innate lymphoid cells type 2 (ILC2s), mast cells, Th2 cells, and eosinophils. This binding triggers a potent cascade of pro-inflammatory cytokines (IL-4, IL-5, and IL-13), leading to airway hyperresponsiveness, mucus hypersecretion, and airway remodeling.[6] Meta-analyses have demonstrated that serum IL-33 levels are significantly elevated in asthmatic children and correlate with disease severity, positioning IL-33 as both a promising biomarker and a potential therapeutic target.[7]

Concurrently, there is growing evidence implicating nutritional status, particularly vitamin D deficiency, in the pathogenesis and exacerbation of childhood asthma. Vitamin D is a potent immunomodulator that regulates both innate and adaptive immune responses.[8] Epidemiological data indicate a pandemic of vitamin D deficiency among children, particularly in the Middle East, where cultural practices, reduced outdoor physical activity, and limited sun exposure contribute to critically low serum 25-hydroxyvitamin D3 levels.[9] Systematic reviews have shown that vitamin D supplementation can significantly reduce the rate of asthma exacerbations requiring systemic corticosteroids.[10] However, the exact molecular mechanisms by which vitamin D deficiency exacerbates asthma remain incompletely understood.

A pivotal yet underexplored mechanistic link between vitamin D and airway inflammation involves regulation of the IL-33/ST2 axis. Experimental *in vitro* studies have demonstrated that vitamin D actively enhances the production of soluble ST2 (sST2), a decoy receptor that binds to free IL-33, thereby neutralizing its pro-inflammatory signaling.[11] Consequently, vitamin D deficiency may reduce sST2, allowing unchecked IL-33 activity and amplifying the inflammatory cascade. Despite this compelling biological rationale, there is a distinct knowledge gap in the clinical literature. A recent comprehensive systematic review of 71 studies investigating the association between vitamin D status and inflammatory biomarkers in asthma noted significant inconsistencies and a lack of sufficient clinical data specifically examining the simultaneous interaction between vitamin D and IL-33 in pediatric cohorts.[12] Furthermore, most existing studies have evaluated these biomarkers in isolation, and the combined diagnostic utility of serum IL-33 and vitamin D-particularly using rigorous Receiver Operating Characteristic (ROC) curve analysis and multivariate predictive modeling-has not been adequately established.

To address this knowledge gap, the present study was designed to investigate the dual profiles of serum IL-33 and vitamin D3 in children with asthma. The novelty of this research extends significantly beyond being the first study of its kind in an Iraqi pediatric population. Specifically, this study aims to: 1) provide clinical validation of the inverse correlation between a potent epithelial alarmin (IL-33) and an immunomodulatory nutrient (vitamin D3) across varying degrees of asthma severity, 2) establish precise, ROC-derived diagnostic cut-off values for both biomarkers to differentiate asthmatic children from healthy controls, and 3) construct a highly accurate multivariate logistic regression model to evaluate the independent predictive value of these biomarkers alongside anthropometric factors such as body mass index (BMI). By bridging the gap between *in vitro* mechanistic theories and *in vivo* clinical realities, this study seeks to provide new scientific insights that could inform targeted, biomarker-guided therapeutic and nutritional interventions for childhood asthma.

Materials and methods

Study design and setting

A case-control study was conducted over 7 months, from July 10, 2025, to February 15, 2026. The study enrolled children attending the outpatient clinics and emergency department of the Children Teaching Hospital in Karbala, Iraq. The research protocol was reviewed and officially approved by the Institutional Ethics Committee at the College of Medicine, University of Kufa, in strict adherence to the ethical principles outlined in the Declaration of Helsinki. Prior to enrollment, written informed consent was obtained from the parents or legal guardians of all participating children.

Study population and sampling

The study comprised 120 pediatric subjects, systematically divided into two age- and gender-matched

groups. The case group included 60 children diagnosed with asthma, while the control group consisted of 60 apparently healthy children with no personal or familial history of allergic or chronic respiratory diseases. The sample size was determined to be statistically adequate and consistent with similar regional case-control studies in Iraq that investigated immunological markers and vitamin D status in pediatric asthma. For instance, Al-Tuama *et al*. used a comparable cohort of 116 subjects (50 asthmatics and 66 controls) in Karbala,[13] Al-Hasnawi and Al-Hasnawi evaluated Interleukins in 149 pediatric subjects[14] and Al-Sharifi *et al*. investigated vitamin D deficiency in 100 Iraqi children.[15]

Inclusion and exclusion criteria

Asthmatic children were diagnosed and classified according to the Global Initiative for Asthma (GINA) guidelines.[16] Disease severity was stratified into mild, moderate, and severe based on clinical presentation, symptom frequency, and the requirement for pharmacological intervention. Children with other chronic systemic illnesses, autoimmune diseases, congenital anomalies, or those currently receiving immunomodulatory therapies (other than standard asthma medications) were excluded from the study to prevent confounding effects on inflammatory biomarkers.

Clinical and demographic data collection

A comprehensive questionnaire was administered via direct interviews with the parents/guardians to collect detailed demographic and clinical data. Anthropometric measurements, including weight and height, were recorded to calculate the BMI. Additional data gathered included passive smoking exposure, residential area (urban vs rural), household pet exposure, and a detailed family history of asthma or atopy. Clinical manifestations (e.g., shortness of breath, wheezing, cough) and specific environmental triggers (e.g., cold air, dust, food allergies) were systematically documented.

Blood sampling and preparation

Approximately 3-5 mL of venous blood was drawn from each participant under aseptic conditions. The whole blood samples were collected into sterile, plain gel tubes and allowed to clot at room temperature for 30 min. Subsequently, the samples were centrifuged at 3000 rpm for 15 min to separate the serum. The extracted serum was carefully aliquoted into sterile Eppendorf tubes and immediately cryopreserved at -20 °C until biochemical analysis, avoiding repeated freeze-thaw cycles that could degrade the target proteins.

Biochemical analysis (ELISA)

The quantitative determination of serum biomarkers was performed using highly sensitive, commercially available enzyme-linked immunosorbent assay (ELISA) kits, strictly following the manufacturer's standard operating procedures. Serum IL-33 and 25-hydroxyvitamin D3 were measured using commercially available ELISA kits according to the manufacturers' instructions. Serum IL-33 was quantified with the human IL-33 ELISA Kit (Cat. No. E0044Hu, Bioassay Technology Laboratory [BT LAB], Zhejiang, China), and serum 25-hydroxyvitamin D3 was assessed using the Human Vitamin D ELISA Kit (Cat. No. E1543Hu, BT LAB, Zhejiang, China). Both assays were based on a quantitative sandwich enzyme immunoassay technique using pre-coated microtiter plates, biotin-conjugated detection antibodies, and streptavidin-horseradish peroxidase, with optical density measured at 450 nm. The assay range and analytical sensitivity were 10-2000 ng/L and 5.36 ng/L for IL-33, and 0.5-200 ng/mL and 0.23 ng/mL for 25-hydroxyvitamin D3, respectively. Assay precision was acceptable for both biomarkers, with intra-assay and inter-assay coefficients of variation of <8% and <10%, respectively. Vitamin D status was classified as sufficient (≥30 ng/mL), insufficient (20.0-29.9 ng/mL), or deficient (<20 ng/mL) according to established pediatric endocrine guidelines.[17]

Statistical analysis

Data management and statistical analyses were executed using the Statistical Package for the Social Sciences (SPSS) software, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normal distribution using the Shapiro-Wilk test. Normally distributed continuous data were presented as Mean±SD and compared using the independent samples t-test for two groups, or One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test for multi-group comparisons (e.g., asthma severity). Categorical variables were expressed as frequencies and percentages (N, %) and analyzed utilizing the Pearson Chi-Square test or Fisher's Exact test, as appropriate.

Bivariate correlations between continuous variables were evaluated using Spearman's rank correlation coefficient (r) given the data's nonnormal distribution. To assess the diagnostic utility of the biomarkers, Receiver Operating Characteristic (ROC) curve analysis was performed, calculating the Area Under the Curve (AUC), sensitivity, specificity, and optimal cut-off values determined via Youden's *J* statistic. A multivariate binary logistic regression model was constructed to identify independent predictors of childhood asthma, reporting adjusted Odds Ratios (aOR) and 95% Confidence Intervals (CI). The model's goodness-of-fit was verified using the Hosmer-Leme show test and Nagelkerke R^2. A two-tailed $p<0.05$ was considered statistically significant, while $p<0.001$ was deemed highly significant.

Results

Table 1 shows that children with asthma and controls were comparable in age and sex (p=0.718 and p=0.708, respectively). However, BMI was significantly higher in the asthma group (19.58±5.73 vs 16.61±2.18 kg/m^2, p=0.001), with overweight/obesity identified only among asthmatic children (15.0%). Passive smoking, positive family history of asthma/atopy, and urban residence were all significantly more frequent in asthmatic children than in controls (60.0% vs 28.3%, p<0.001, 53.3% vs 13.3%, p<0.001, and 70.0% vs 50.0%, p=0.040, respectively), while pet exposure was comparable between groups (p=0.265). All respiratory symptoms and triggers were significantly more common in the asthma group (all p<0.001). Asthma severity was mild in 33.3%, moderate in 40.0%, and severe in 26.7% of cases.

Table 1. Comparison of demographic and clinical characteristics between asthma patients and healthy controls.

Parameter	**Asthma** (N=60)	**Control** (N=60)	***p* value**
Age (years), Mean±SD	9.27±2.79	9.53±3.16	0.718 (NS)
• 5-8	26 (43.3%)	26 (43.3%)	
• 9-11	18 (30.0%)	15 (25.0%)	
• 12-15	16 (26.7%)	19 (31.7%)	
Gender, N (%)			0.708 (NS)
• Male	38 (63.3%)	35 (58.3%)	
• Female	22 (36.7%)	25 (41.7%)	
BMI (kg/m^2), Mean±SD	19.58±5.73	16.61±2.18	0.001**
• Underweight (<18.5)	35 (58.3%)	51 (85.0%)	
• Normal (18.5-24.9)	16 (26.7%)	9 (15.0%)	
• Overweight (25-29.9)	5 (8.3%)	0 (0.0%)	
• Obese (≥30)	4 (6.7%)	0 (0.0%)	
Passive smoking exposure	36 (60.0%)	17 (28.3%)	<0.001***
Pet exposure	12 (20.0%)	7 (11.7%)	0.265 (NS)
Urban residence	42 (70.0%)	30 (50.0%)	0.040*
Family history of asthma	32 (53.3%)	8 (13.3%)	<0.001***
Clinical manifestations, N (%)			
• Shortness of breath	54 (90.0%)	0 (0.0%)	<0.001***
• Wheezing	37 (61.7%)	0 (0.0%)	<0.001***
• Cough	44 (73.3%)	0 (0.0%)	<0.001***
Food allergy	27 (45.0%)	3 (5.0%)	<0.001***
Triggers, N (%)			
• Cold air	46 (76.7%)	0 (0.0%)	<0.001***
• Dust	45 (75.0%)	0 (0.0%)	<0.001***
Asthma severity, N (%)			
• Mild	20 (33.3%)	NA	
• Moderate	24 (40.0%)	NA	
• Severe	16 (26.7%)	NA	

Note: *NS: not significant at p>0.05), * p<0.05, **p<0.01; ***p<0.001, NA: not applicable. Continuous variables are expressed as Mean±SD and compared using the independent samples t-test. Categorical variables are expressed as N (%) and compared using the Chi-Square test or Fisher's Exact test.*

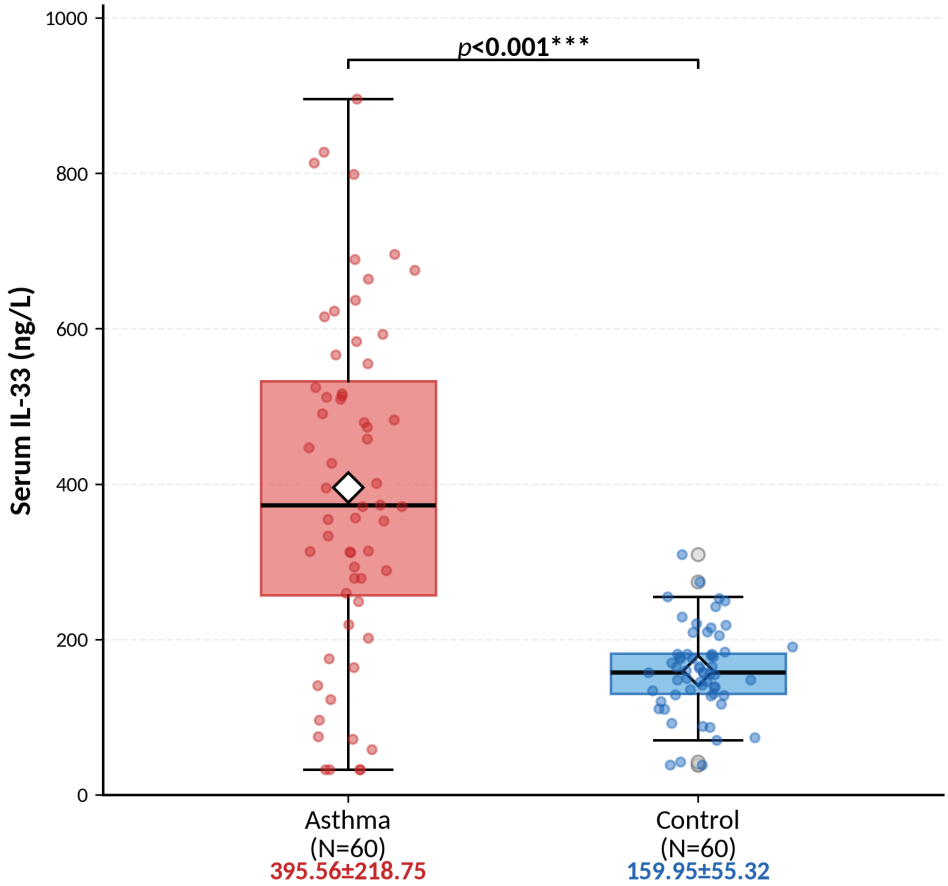


Figure 1. *Comparison of IL-33 levels between asthma patients (N=60) and healthy controls (N=60). Box plots show median (horizontal line), mean (diamond), interquartile range (box), and individual data points. The numbers represent Mean±SD.*

Comparison of serum biomarker levels between asthma patients and healthy controls

Table 2, Figures, and Figure 2 showed marked differences in serum biomarker levels between asthmatic children and controls. Serum IL-33 was significantly higher in the asthma group than in controls (395.56 218.75 vs 159.95±55.32 ng/L, $p<0.001$), whereas serum 25(OH)D3 was significantly lower (17.01±7.38 vs 28.20±10.60 ng/mL, $p<0.001$). These findings indicate an enhanced inflammatory profile and reduced vitamin D status in children with asthma.

Table 2. Serum biomarker levels in asthma patients and healthy controls.

Parameter	**Asthma** (N=60)	**Control** (N=60)	***p* value**
IL-33 (ng/L)	395.56±218.75	159.95±55.32	<0.001***
25-Hydroxyvitamin D3 (vitamin D3, ng/L)	17.01±7.38	28.20±10.60	<0.001***

Note: *Data are expressed as Mean±SD. A statistical comparison was performed using the independent-samples t-test. ***p<0.001 indicates a highly significant difference.*

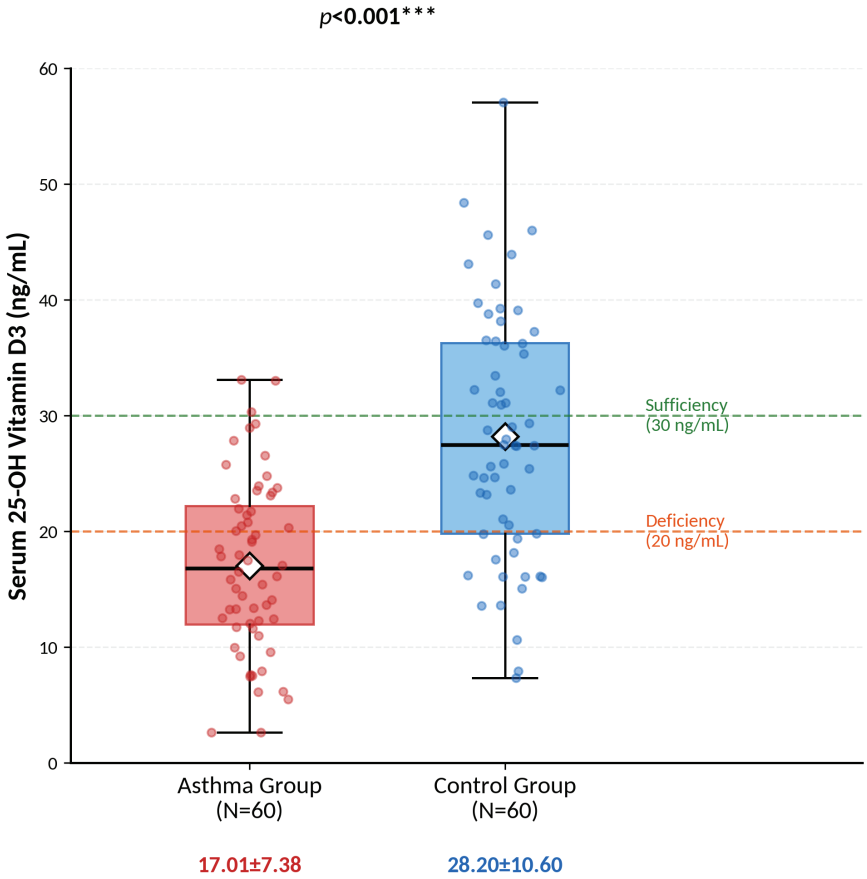


Figure 2. *Comparison of serum 25-hydroxyvitamin D3 levels between asthma patients (N=60) and healthy controls (N=60). Box plots show median (horizontal line), mean (diamond), interquartile range (box), and individual data points. Dashed lines indicate vitamin D deficiency (<20 ng/mL) and sufficiency (≥30 ng/mL) cutoffs. The numbers represent Mean±SD.*

Table 3, Figures 3, and Figure 4 showed significant associations between serum biomarkers and asthma severity. IL-33 increased progressively across mild, moderate, and severe asthma (276.2±87.6, 386.7±79.3, and 595.5±132.9 ng/L, respectively, $p<0.001$), while 25(OH)D3 decreased significantly with increasing severity (20.7±4.4, 17.5±5.6, and 11.0±3.8 ng/mL, respectively, $p<0.001$). Pairwise comparisons confirmed significant differences between severity groups for both markers, indicating that higher IL-33 and lower vitamin D levels were associated with more severe asthma.

Table 3. Comparison of serum biomarker levels among asthmatic children classified by disease severity.

Biomarker	**Mild** (N=20)	**Moderate** (N=24)	**Severe** (N=16)	***p* value**
Serum IL-33 (ng/L)	276.2±87.6A	386.7±79.3B	595.5±132.9C	<0.001***
25(OH)D3 (ng/mL)	20.7±4.4A	17.5±5.6B	11.0±3.8C	<0.001***

Note: *Data presented as mean±SD. Different letters (A/B/C) indicate significant pairwise differences (one-way ANOVA followed by Tukey's post hoc test, p<0.05); ***p<0.001 indicates a highly significant difference among severity groups, A, B, C: significant pairwise differences (one-way ANOVA followed by Tukey's post hoc test).*

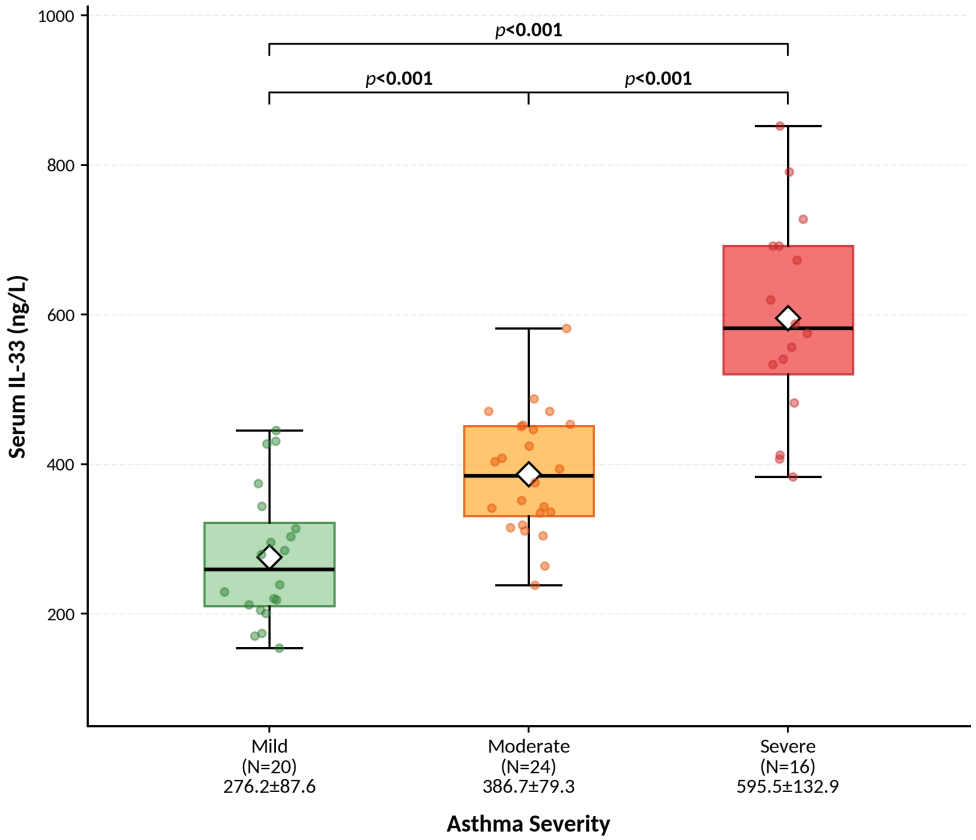


Figure 3. *Comparison of serum IL-33 levels (ng/L) among asthmatic children classified by disease severity. IL-33 levels increased progressively with severity (p<0.001 for all pairwise comparisons).*

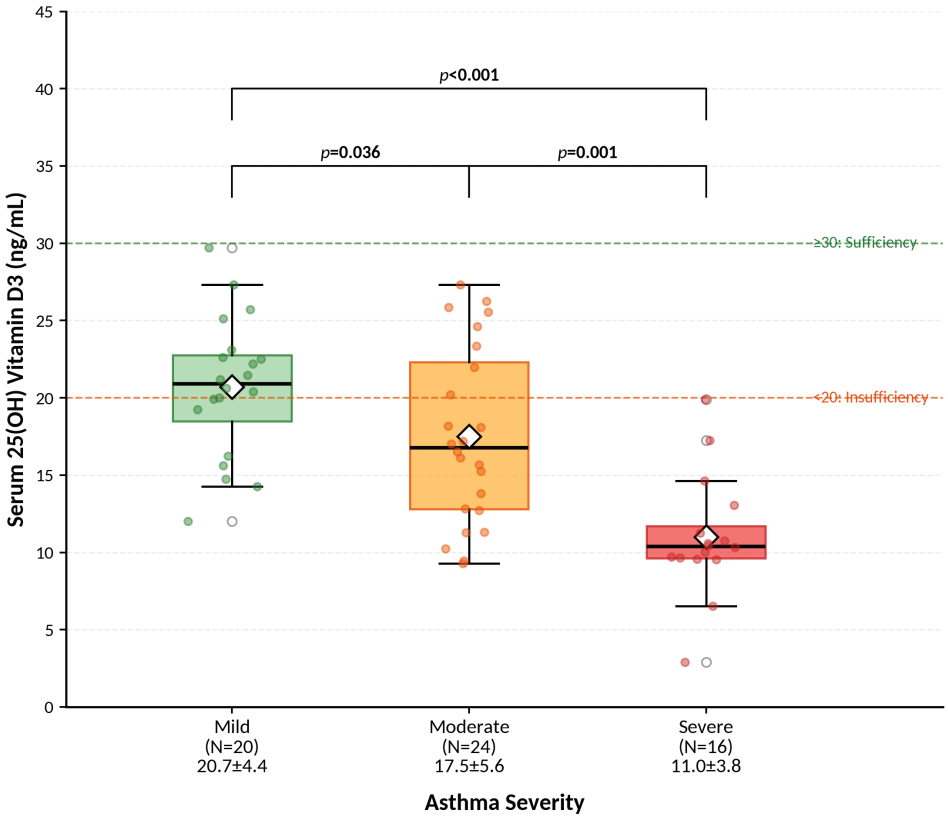


Figure 4. *Comparison of serum 25-hydroxyvitamin D3 levels (ng/mL) among asthmatic children classified by disease severity. Dashed lines indicate clinical thresholds for vitamin D insufficiency (<20 ng/mL) and sufficiency (≥30 ng/mL). Vitamin D3 showed an inverse relationship with severity (p=0.036 for Mild vs Moderate, p=0.001 for Moderate vs Severe, p<0.001 for Mild vs Severe).*

Spearman correlation matrix between serum biomarkers in asthmatic children

Table 4 and Figure 5 showed a significant inverse correlation between serum IL-33 and 25(OH)D3 levels in asthmatic children (r=-0.614, p<0.001). Higher IL-33 levels were associated with lower vitamin D concentrations, supporting an inverse relationship between inflammatory activity and vitamin D status in childhood asthma.

Table 4. Spearman's rank correlation coefficient (r) between inflammatory biomarkers and vitamin D3 among asthmatic children (N=60).

Biomarkers	Serum IL-33	Serum vitamin D3
Serum IL-33 (ng/L)	1.000	-0.614***
Serum vitamin D3 (ng/mL)	-0.614***	1.000

Note: *Data are presented as Spearman's rank correlation coefficient (r), ***p<0.001 indicates a highly significant correlation.*

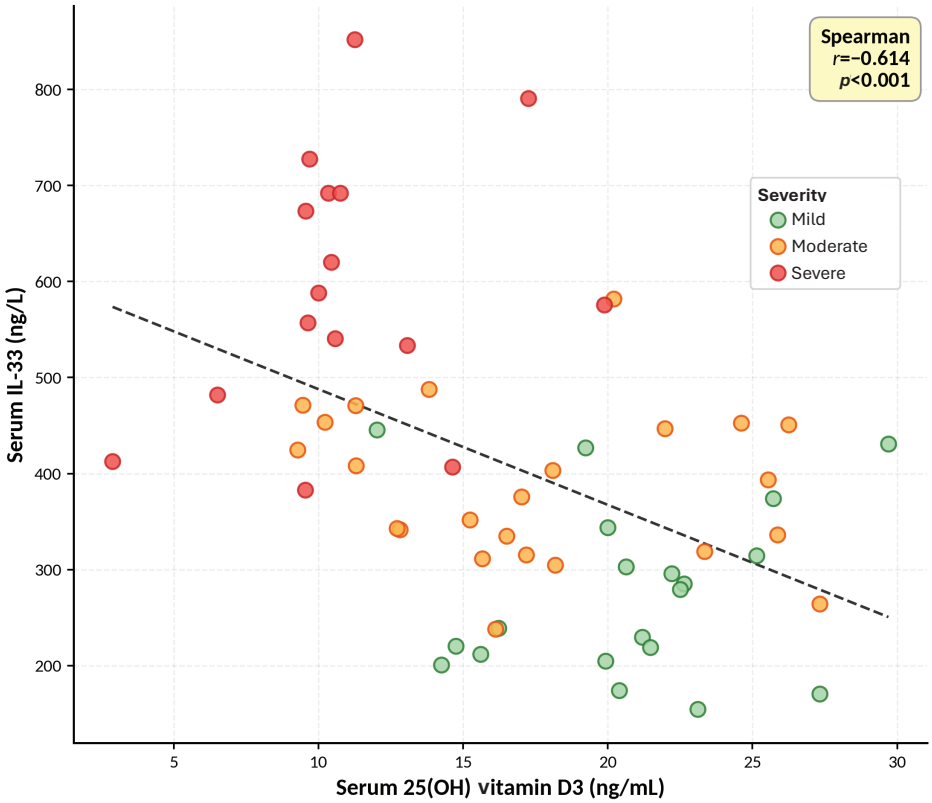


Figure 5. *Scatter plot showing the inverse correlation between serum 25(OH) vitamin D3 (ng/mL) and serum IL-33 (ng/L) in asthmatic children (N=60). Color-coded by disease severity. Spearman correlation coefficient r=-0.614, p<0.001. This negative correlation is consistent with vitamin D's immunomodulatory role in suppressing epithelial alarmin production.*

Table 5 and Figure 6 showed that both IL-33 and 25(OH)D3 significantly distinguished asthmatic children from healthy controls (both p<0.001). IL-33 demonstrated superior diagnostic performance, with an AUC of 0.933 (95% CI: 0.874-0.978) and an optimal cutoff of ≥239.4 ng/L, yielding 85.0% sensitivity and 93.3% specificity. In comparison, 25(OH)D3 showed a lower but still significant diagnostic value, with an AUC of 0.819 (95% CI: 0.734-0.890) and an optimal cutoff of ≤20.3 ng/mL, corresponding to 80.0% sensitivity and 75.0% specificity.

Table 5. Diagnostic performance of biomarkers in differentiating asthma patients from healthy controls (ROC curve analysis).

Diagnostic performance	Biomarker	
	Serum IL-33 (ng/L)	Serum vitamin D3 (ng/mL)
Cut-off	≥239.4	≤20.3
AUC (95% CI)	0.933 (0.874-0.978)	0.819 (0.734-0.890)
p value	<0.001***	<0.001***
Sensitivity (%)	85.0	85.0
Specificity (%)	93.3	75.0
PPV (%)	92.7	76.2
NPV (%)	86.2	78.9
Accuracy (%)	89.2	77.5
Youden's *J*	0.783	0.550

Note: *ROC: receiver operating characteristic, AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value. Cut-off values were determined using Youden's J statistic. For vitamin D3, values below the cut-off indicate asthma, and for IL-33, values above the cut-off indicate asthma.*

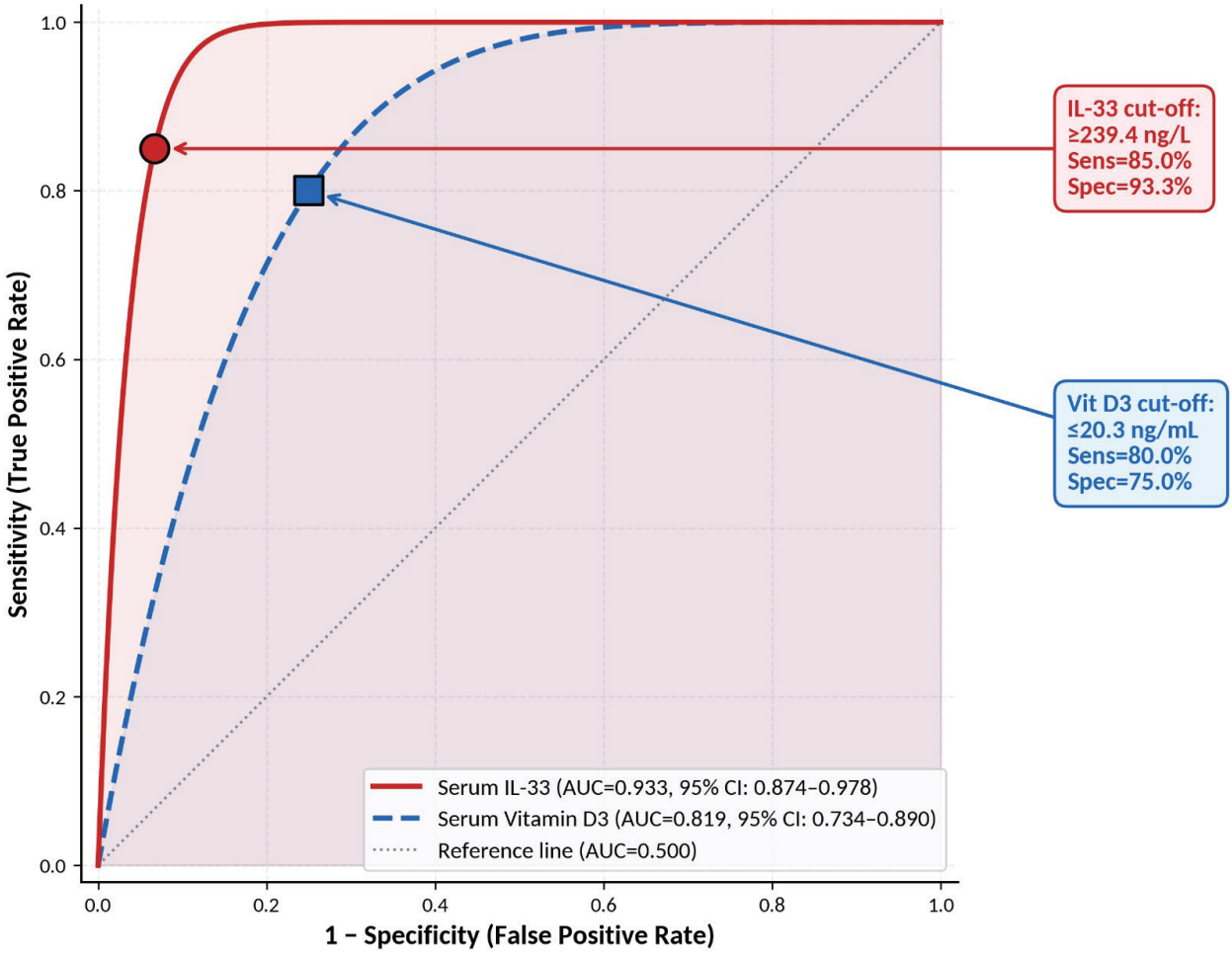


Figure 6. *ROC curve analysis of serum IL-33 and vitamin D3 for the diagnosis of childhood asthma.*

Binary logistic regression analysis of independent predictors of childhood asthma

Table 6 and Figure 7 showed that BMI and serum IL-33 were independent predictors of childhood asthma in the multivariable logistic regression model. Higher BMI was associated with increased odds of asthma (aOR=1.528, 95% CI: 1.089-2.144, *p*=0.014), and higher IL-33 was also significantly associated with asthma risk (aOR=1.015, 95% CI: 1.002-1.027, *p*=0.020). Although higher 25(OH)D3 was associated with lower odds of asthma, this association was not statistically significant (aOR=0.919, *p*=0.097). The model showed excellent performance, with good calibration, high discriminatory ability (AUC=0.972), and an overall accuracy of 95.8%.

Table 6. Binary logistic regression model for independent predictors of childhood asthma.

Panel A. Final parsimonious logistic regression coefficients.

Predictor	B	SE	Wald	aOR	95% CI	*p* value	Interpretation
BMI (kg/m^2)	0.424	0.173	6.02	1.528	1.089-2.144	0.014*	Each 1 kg/m^2 increase: 52.8% higher asthma odds.
Serum IL-33 (ng/L)	0.015	0.006	5.38	1.015	1.002-1.027	0.020*	Each 10 ng/L increase: 16.1% higher asthma odds.
Serum vitamin D3 (ng/mL)	-0.084	0.051	2.75	0.919	0.832-1.015	0.097	Protective trend; 8.1% lower odds per 1 ng/mL, NS.

Panel B. Model summary and goodness-of-fit.

Metric	Value	Interpretation
-2 Log-Likelihood	40.63	Lower values indicate better fit in comparative models.
Chi-square (model)	125.72, *p*<0.001	Overall model was highly significant.
Cox & Snell R^2	0.649	About 64.9% of variance explained.
Nagelkerke R^2	0.866	About 86.6% of variance explained.
Overall classification accuracy	95.8% (115/120)	Excellent overall classification performance.
Sensitivity	93.3% (56/60)	Strong case-detection ability.
Specificity	98.3% (59/60)	Excellent control identification.
AUC (ROC)	0.972	Outstanding discriminative ability.
Hosmer-Leneshow test	chi-square=8.95, *p*=0.176	Adequate calibration and good model fit.

Note: *B: regression coefficient, SE: standard error, AOR: adjusted odds ratio, CI: confidence interval. *p<0.05.*

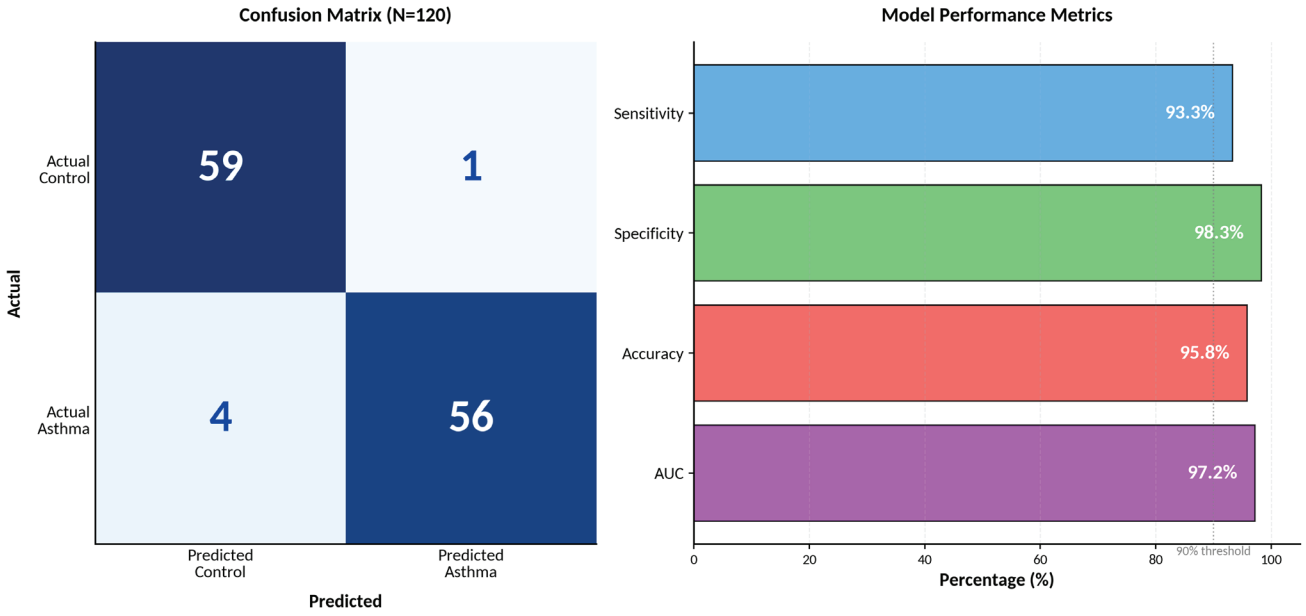


Figure 7. *Classification performance of the final multivariate logistic regression model. Left panel: confusion matrix, Right panel: model performance metrics (sensitivity, specificity, accuracy, AUC).*

Effect of gender on serum biomarker levels in asthmatic children

Table 7 showed a significant sex-related difference in serum 25(OH)D3 levels among asthmatic children, with males having lower levels than females (13.79±5.36 vs. 21.51±5.15 ng/mL, $p<0.001$), with a large effect size (r=0.609). In contrast, serum IL-33 levels were higher in males than females, but the difference was not statistically significant (428.91±156.41 vs 365.70±132.17 ng/L, $p=0.0858$), with a small effect size (r=0.222).

Table 7. Comparison of biomarker levels between male and female asthmatic children (N=60).

Parameter	**Males** (N=38)	**Females** (N=22)	p value	**Effect size** (r)	Significance
IL-33 (ng/L)	428.91±156.41	365.70±132.17	0.0858	0.222 (small)	NS
Vitamin D3 (ng/mL)	13.79±5.36	21.51±5.15	<0.001	0.609 (large)	***

Note: *Data presented as Mean±SD, NS: not significant, ***p<0.001 = highly significant.*

Discussion

The present study provides compelling evidence for the interplay between systemic inflammation, as reflected by elevated serum IL-33, and nutritional deficiency, manifested by reduced serum 25-hydroxyvitamin D3, in the pathogenesis and clinical severity of childhood asthma. To the best of our knowledge, this is one of the first studies conducted in Iraq to simultaneously investigate both biomarkers in a pediatric asthmatic population and to evaluate their combined diagnostic and predictive utility through ROC curve analysis and multivariate logistic regression modeling.

Demographic and environmental risk factors

The demographic analysis revealed that the asthma and control groups were well-matched for age and sex, thereby minimizing confounding and strengthening the internal validity of the comparative analyses. However, several modifiable and non-modifiable risk factors were significantly associated with the asthma phenotype in our cohort.

BMI was significantly higher in asthmatic children than in healthy controls (19.58±5.73 vs 16.61±2.18 kg/m^2, $p=0.001$), with 15.0% of the asthma group classified as overweight or obese, compared to none in the control group. This finding is consistent with the large-scale meta-analysis by Deng *et al.*, which demonstrated that overweight and obesity are significant risk factors for childhood asthma and wheeze across 18 studies involving 73,252 children.[18] Similarly, Azizpour *et al.* confirmed this association in their systematic review and meta-analysis of case-control studies, reporting a significant positive relationship between elevated BMI and asthma risk in children.[19] The mechanistic basis for this association involves adipose tissue-derived pro-inflammatory cytokines (adipokines), including leptin and TNF-alpha, which promote systemic inflammation and airway hyperresponsiveness, thereby predisposing obese children to asthma development and exacerbation.[20]

Notably, the relationship between BMI, IL-33, vitamin D, and asthma is recognized to vary across pediatric age strata and between sexes. Adiposity-related inflammation may exert distinct effects across early childhood, pre-pubertal, and adolescent periods, and pubertal changes substantially modulate vitamin D status, particularly in adolescent boys and in regions with high baseline deficiency, such as the Middle East.[9] A formal age- and gender-stratified evaluation of these biomarkers across the three age subgroups (5-8, 9-11, and 12–15 years) was beyond the statistical power of the present cohort and is acknowledged in the Limitations section as an important direction for future, adequately powered studies.

Passive smoking exposure was markedly more prevalent among asthmatic children (60.0%) compared to controls (28.3%, p<0.001). This observation aligns with the comprehensive meta-analysis conducted by He *et al.,* which demonstrated a robust positive association between both prenatal and postnatal secondhand smoke exposure and the occurrence of childhood asthma.[21] Environmental tobacco smoke contains numerous irritants and oxidative agents that damage the airway epithelium, enhance mucus secretion, and promote Th2-mediated inflammatory responses, all of which are central to asthma pathophysiology.[1]

A positive family history of asthma or atopy was reported in 53.3% of asthmatic children compared to only 13.3% of controls (p<0.001). This strong familial predisposition is well-documented in the literature. Ober and Yao reported heritability estimates for asthma ranging between 35% and 95%, underscoring the substantial genetic contribution to disease susceptibility.[22] Bjerg *et al*. further demonstrated in a large pediatric cohort that a family history of asthma and atopy significantly increases the risk of developing these conditions in offspring, even after adjusting for known environmental risk factors.[23]

Urban residence was significantly associated with asthma in our study (70.0% vs 50.0%, p=0.040). This finding is supported by the extensive review by Grant *et al*., which concluded that children living in urban areas are at higher risk of developing asthma due to increased exposure to traffic-related air pollution, indoor allergens, and reduced green space.[24] Aligne *et al*. similarly identified urban residence as an independent risk factor for childhood asthma after controlling for race, poverty, and other environmental variables.[25]

Serum IL-33 levels

The central finding of this study is the markedly elevated serum IL-33 levels in asthmatic children compared to healthy controls (395.56±218.75 ng/L vs 159.95±55.32 ng/L, p<0.001). This result agrees with several Iraqi studies. Shaban *et al*., in a study conducted in Iraq, reported that serum IL-33 was significantly up-regulated in asthma patients compared to controls, further supporting the role of this alarmin cytokine in the local asthmatic population.[26] Additionally, Al-Hasnawi and Al-Hasnawi investigated IL-25 and IL-33 as immunological markers in Iraqi pediatric asthma patients and confirmed the elevation of both Interleukins in the asthma group.[14]

Internationally, our findings are corroborated by the landmark study of Bahrami Mahneh et al. conducted in Iran, which demonstrated significantly higher serum IL-33 levels in 61 asthmatic children compared to 63 age- and gender-matched controls (15.17±32.3 vs 0.61±2.16 pg/ml, p=0.028).[27] Although the absolute concentrations differ due to variations in ELISA kits and assay methodologies, the directional trend is identical. Wang *et al*. further consolidated this evidence through a systematic review and meta-analysis, confirming that serum IL-33 is consistently elevated in children with asthma across multiple populations.[7]

Furthermore, the progressive increase in IL-33 levels corresponding to asthma severity observed in our study (mild: 276.2±87.6, moderate: 386.7±79.3, severe: 595.5±132.9 ng/L, p<0.001) mirrors the severity-dependent pattern reported by Bahrami Mahneh et al. (2016), who documented IL-33 concentrations of 9.92, 13.68, and 31.92 pg/ml in mild, moderate, and severe asthma, respectively (p=0.026).[27] Yang *et al*. also confirmed that serum IL-33 is elevated in asthmatic children and increases in proportion to the severity of pulmonary ventilation obstruction.[28] These converging findings across diverse populations strongly support the utility of IL-33 as a biomarker of disease activity and severity in childhood asthma.

Serum vitamin D3 levels

Serum 25-hydroxyvitamin D3 levels were significantly lower in asthmatic children compared to controls (17.01±7.38 vs 28.20±10.60 ng/mL, p<0.001), with the asthma group's mean falling below the clinical deficiency threshold of 20 ng/mL. This finding is particularly relevant in the Iraqi context. Al-Tuama *et al*., in a study conducted at the same hospital setting in Karbala, Iraq, reported a significant association between vitamin D deficiency and childhood asthma, with asthmatic children demonstrating substantially lower vitamin D levels than their healthy counterparts.[13] Al-Sharifi *et al*. similarly concluded that vitamin D deficiency is very common among Iraqi asthmatic children and that vitamin D inadequacy is predominant in asthmatic children living in Iraq.[15]

At the international level, our results are consistent with the large case-control study by Bener *et al*. in Qatar, which identified vitamin D deficiency as a strong predictor of asthma in children, with most asthmatic children exhibiting vitamin D deficiency compared to controls.[29] Esfandiar *et al*. also demonstrated a significant inverse relationship between vitamin D levels and asthma severity in children in an Iranian study.[30] The dose-dependent decline in vitamin D

levels with increasing asthma severity observed in our study (mild: 20.7±4.4, moderate: 17.5±5.6, severe: 11.0±3.8 ng/mL, $p<0.001$) is particularly noteworthy, as it indicates that severe asthmatic children are in a state of critical vitamin D deficiency, potentially exacerbating their inflammatory burden.

Inverse correlation between IL-33 and vitamin D: mechanistic insights

A key finding of this study is the strong inverse correlation between serum IL-33 and vitamin D3 levels (Spearman r=-0.614, $p<0.001$). This relationship provides important mechanistic insights into the immunopathology of childhood asthma. The biological plausibility of this inverse association is well-supported by the seminal work of Pfeffer *et al.,* who demonstrated that vitamin D enhances the production of soluble ST2 (sST2), a decoy receptor that sequesters IL-33 and inhibits its pro-inflammatory signaling.[11] Specifically, vitamin D selectively upregulates the expression of sST2 mRNA in human bronchial epithelial cells and $CD4^+$ T lymphocytes, and conditioned medium from vitamin D-treated epithelial cells significantly inhibited IL-33-induced mast cell activation.[11] This mechanism provides a direct molecular link between vitamin D deficiency and the unchecked elevation of IL-33 observed in our asthmatic cohort.

IL-33, a member of the IL-1 cytokine family, functions as an epithelial-derived alarmin that is released upon cellular damage or stress. As described by Cayrol and Girard, IL-33 activates group 2 innate lymphoid cells (ILC2s), inducing the production of large amounts of IL-5 and IL-13, which are central mediators of type 2 inflammation, eosinophilic infiltration, mucus hypersecretion, and airway remodeling.[6] Duchesne *et al*. further elaborated that IL-33 released from bronchial epithelial cells serves as a frontline mediator of the asthmatic inflammatory response, bridging innate and adaptive immunity.[4]

Rai *et al*. provided additional evidence that vitamin D acts as an immunomodulator that regulates IL-33 secretion and macrophage phenotypic polarization, suggesting that adequate vitamin D status may serve as a natural brake on the IL-33/ST2 inflammatory axis.[31] Bonanno *et al*. also reported an inverse correlation between 25-hydroxyvitamin D levels and IL-33 in children with allergic airway disease, further supporting the biological relevance of our findings.[32]

Diagnostic utility of IL-33 and vitamin D3

The ROC curve analysis revealed that serum IL-33 possesses excellent diagnostic accuracy for childhood asthma, with an AUC of 0.933 (95% CI: 0.874-0.978), a sensitivity of 85.0%, and a specificity of 93.3% at an optimal cut-off of ≥239.4 ng/L. This diagnostic performance substantially exceeds that reported by Shaban *et al*. in Iraq, who found a sensitivity of 60.6% and specificity of 59.5% at a cut-off of 68.3 pg/mL.[26] The superior performance in our study may be attributed to differences in ELISA kit sensitivity, patient selection criteria, and the larger proportion of moderate-to-severe cases in our cohort. Yang *et al*. demonstrated that combining IL-33 with other markers, such as FEF75% z-score and FeNO, can further improve diagnostic sensitivity to 90.1%, suggesting that multi-marker panels may offer even greater clinical utility.[28]

Serum vitamin D3 also demonstrated good diagnostic value (AUC=0.819, 95% CI: 0.734-0.890), with an optimal cut-off of ≤20.3 ng/mL closely aligning with the established clinical threshold for vitamin D deficiency (<20 ng/mL). This convergence between the statistically derived cut-off and the clinically recognized deficiency threshold enhances the translational relevance of our findings and supports the integration of vitamin D assessment into routine asthma evaluation protocols.

Independent predictors of childhood asthma

The multivariate binary logistic regression analysis identified BMI (aOR=1.528, 95% CI: 1.089-2.144, $p=0.014$) and serum IL-33 (aOR=1.015 per 1 ng/L increase, $p=0.020$) as significant independent predictors of childhood asthma. The identification of BMI as an independent predictor is consistent with the findings of Fang *et al*., who reported that a one-unit increase in BMI was associated with 4% higher odds of asthma in children,[33,] and with those of Li *et al*., who confirmed that high BMI independently predicts increased asthma occurrence.[34]

Although serum vitamin D3 showed a protective trend (aOR=0.919), it did not reach statistical significance in the multivariate model ($p=0.097$). This may be explained by the strong collinearity between vitamin D and IL-33, as evidenced by their robust inverse correlation (r=-0.614). When both variables are included simultaneously in a regression model, the dominant variance explained by IL-33 may attenuate the independent contribution of vitamin D. Nevertheless, the overall model demonstrated outstanding performance, with a Nagelkerke R^2 of 0.866, an overall classification accuracy of 95.8%, and an AUC of 0.972, confirming the excellent predictive capacity of the combined biomarker panel.

Gender disparities in biomarker levels

An important secondary finding of this study is the significant gender-based disparity in vitamin D levels among asthmatic children. Males exhibited substantially lower vitamin D concentrations compared to females (13.79±5.36 vs 21.51±5.15 ng/mL, $p<0.001$), with a large effect size (r=0.609). This finding is consistent with the study by Li *et al*., who reported that males with two or more allergic diseases had significantly lower vitamin D levels than their female counterparts.[35] Hollams *et al*. in a large Australian cohort, also found that males with inadequate vitamin D at ages

6 and 14 years had increased atopy and bronchial hyperresponsiveness.[36]

The gender disparity in vitamin D levels may be attributed to several factors, including differences in outdoor activity patterns, clothing habits (particularly in Middle Eastern societies where cultural practices may differ between genders), dietary intake, and hormonal influences on vitamin D metabolism. This finding has important clinical implications, suggesting that male asthmatic children may represent a particularly vulnerable subgroup requiring targeted vitamin D screening and supplementation strategies.

In contrast, serum IL-33 levels did not differ significantly between male and female asthmatic children (p=0.0858), with only a small effect size (r=0.222). This suggests that the inflammatory burden, as measured by IL-33, is primarily driven by disease-related factors rather than gender-specific biological differences.

Clinical implications

The findings of this study carry several important clinical implications. Firstly, the strong diagnostic performance of serum IL-33 (AUC=0.933) supports its potential utility as a non-invasive biomarker for diagnosing childhood asthma, particularly in settings where pulmonary function testing may be challenging in young children. Secondly, the significant inverse correlation between vitamin D and IL-33 suggests that vitamin D supplementation may be a viable adjunctive therapeutic strategy to modulate the inflammatory response in asthmatic children. Pfeffer and Hawrylowicz reviewed the evidence supporting a role for vitamin D in secondary prevention, with a focus on reducing asthma exacerbations and inflammation.[37] However, the VDKA randomized clinical trial by Forno *et al*. found that vitamin D3 supplementation did not significantly improve time to severe asthma exacerbation in children with low vitamin D levels, highlighting the need for further investigation into optimal dosing, timing, and patient selection for vitamin D interventions.[38] Thirdly, the identification of BMI as an independent predictor of asthma reinforces the importance of weight management programs as part of comprehensive asthma care in children. Lastly, the pronounced vulnerability of male asthmatic children to severe vitamin D deficiency warrants gender-stratified clinical assessments and targeted nutritional interventions.

Strengths and limitations

This study has several notable strengths. It is one of the first studies in Iraq to simultaneously evaluate both IL-33 and vitamin D in pediatric asthma within a single cohort, enabling direct correlation analysis. The comprehensive statistical approach, including ROC curve analysis with Youden's *J* statistic optimization and multivariate logistic regression with a detailed goodness-of-fit assessment, yields robust, clinically translatable findings. The inclusion of effect size calculations alongside p values enhances the interpretability of the results beyond mere statistical significance. Furthermore, the age- and sex-matching of the study groups minimizes potential confounding.

However, several limitations should be acknowledged. Firstly, the cross-sectional case-control design precludes establishing causal relationships between the biomarkers and asthma. Longitudinal prospective studies are needed to determine whether elevated IL-33 and reduced vitamin D precede asthma onset or are consequences of the disease process. Secondly, the sample size (N=120), while consistent with comparable regional studies, may limit the generalizability of the findings to the broader Iraqi pediatric population. Thirdly, the study did not assess other potential confounders such as dietary habits, sun exposure duration, physical activity levels, or concurrent medication use, which may influence both vitamin D status and inflammatory markers. Fourthly, although the cohort spanned three age subgroups (5-8, 9-11, and 12-15 years) and both genders, the study was not originally powered for age- and gender-stratified subgroup analyses of BMI, IL-33, and vitamin D within the asthma group. With per-subgroup sizes ranging from 16 to 38 asthmatic children, formal stratified statistical comparisons would be underpowered and at high risk of yielding unstable estimates. Accordingly, age- and sex-stratified evaluations of these biomarkers-including age-specific identification of subgroups with the most pronounced IL-33 elevation and vitamin D deficiency-represent an important direction for future, adequately powered, prospectively designed studies. Fifthly, soluble ST2 (sST2) was not measured in our cohort. Although the proposed mechanistic link between vitamin D deficiency and unrestrained IL-33 activity is grounded in the previously reported finding that vitamin D enhances sST2 production *in vitro,*[11] this pathway was inferred rather than directly tested in our participants. Future work should incorporate sST2 measurement from stored sera, where available, to directly validate the vitamin D → sST2 → IL-33 axis in this population. Sixthly, asthma severity was classified based on clinical presentation, symptom frequency, and pharmacological requirements in accordance with GINA guidelines without spirometric confirmation (FEV_1 and FEV_1/FVC ratio).[16] The absence of objective lung function testing may limit the precision of severity stratification and the robustness of dose-response analyses across severity groups. We acknowledge that integrating spirometry-based indices into future studies, particularly in older children able to perform reliable maneuvers, would strengthen severity classification and enable more refined biomarker-lung function correlations. Lastly, the single-center design may introduce selection bias, and multi-center studies across different Iraqi governorates would strengthen

the external validity of these findings.

Future directions

Future research should prioritize large-scale, multi-center prospective cohort studies to establish the temporal relationship between IL-33, vitamin D, and asthma development in Iraqi children. Randomized controlled trials evaluating the efficacy of vitamin D supplementation in reducing IL-33 levels and improving asthma outcomes in this population are warranted. Additionally, investigating the combined diagnostic utility of IL-33 with other biomarkers (such as FeNO, IgE, and eosinophil counts) through multi-marker panel approaches may further enhance diagnostic precision. Genetic studies exploring polymorphisms in the IL1RL1 (ST2) and VDR (vitamin D receptor) genes in the Iraqi population could provide valuable insights into the genetic susceptibility underlying the observed biomarker associations.

Finally, gender-stratified intervention studies targeting vitamin D supplementation in male asthmatic children are recommended to address the pronounced deficiency observed in this subgroup.

Conclusion

This study provides compelling clinical evidence elucidating the intertwined roles of the epithelial alarmin IL-33 and vitamin D3 in the pathogenesis and severity of childhood asthma. Our findings demonstrate a robust, inverse relationship between elevated systemic IL-33 levels and profound vitamin D3 deficiency, both of which correlate significantly with escalating disease severity. The exceptional diagnostic performance of IL-33, alongside the independent predictive value of both IL-33 and elevated BMI, underscores the multifaceted nature of asthma exacerbations. Furthermore, the pronounced vulnerability of male asthmatic children to severe vitamin D deficiency highlights the necessity for individualized clinical assessments. Ultimately, these results bridge the gap between *in vitro* immunological mechanisms and clinical phenotypes, suggesting that targeted interventions-such as biomarker-guided anti-IL-33 therapies combined with gender-stratified vitamin D supplementation and weight management-could offer a synergistic approach to improving disease control and mitigating the inflammatory burden in pediatric asthma.

Ethical approval

The study protocol was reviewed and approved by the Institutional Ethics Committee at the College of Medicine, University of Kufa, Iraq (No: MEC-103, Dated October,2025). The research was conducted in strict adherence to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

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Conflict of interests

The authors declare no competing interests.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos).

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to institutional privacy policies regarding patient data, but are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Zahraa Hazim Aziz: conceptualization, methodology, investigation, data collection, formal analysis, writing: original draft and visualization; **Thikra Abdullah Mahmood:** supervision, validation, methodology, data interpretation, writing: review and edit, project administration.

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