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ORIGINAL RESEARCH

Serum Levels of IL-6, IL-10, TNF-α, and Vitamin D: Their Correlation with Acute Otitis Media in Short-Stature Pediatric Patients

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Introduction: Short stature in children is a global health issue often linked to micronutrient deficiencies like vitamin D, potentially impairing immune function. Acute Otitis Media (AOM) is a common complication of upper respiratory infections in children, with inflammatory cytokines such as IL-6, IL-10, and TNF-α playing a crucial role in its pathogenesis. This study aims to explore the correlation between serum levels of these biomarkers and AOM occurrence in short-stature pediatric patients.

Purpose: To assess the relationship between serum levels of IL-6, IL-10, TNF-α, and vitamin D and their association with AOM in children with short stature.

Patients and Methods: A case-control study was conducted with 180 children aged 24–59 months, divided into four groups: short stature with AOM, short stature without AOM, normal stature with AOM, and normal stature without AOM. Serum levels of IL-6, IL-10, TNF-α, and vitamin D were measured using the ELISA method. The data were analyzed using descriptive, bivariate, and multivariate statistical methods.

Results: Children with short stature and AOM had the highest median levels of IL-6 (12.15 pg/mL) and TNF-α (162.52 pg/mL), while IL-10 levels were lowest in this group (0.56 pg/mL), indicating a robust inflammatory response associated with AOM. Multivariate analysis revealed that male sex, vitamin D levels ≤18.9 ng/mL, IL-6 levels >9.42 pg/mL, and IL-10 levels ≤0.76 pg/mL were significant risk factors for developing AOM, with respective odds ratios indicating markedly elevated risks.

Conclusion: This study underscores the significant role of inflammatory markers and vitamin D deficiency in the development of AOM in short-stature pediatric patients. Regular monitoring and targeted interventions may help prevent AOM in this vulnerable population, warranting future research on the effectiveness of vitamin D supplementation and other therapeutic strategies.

Keywords: acute otitis media, AOM, IL-6, TNF-α, inflammatory cytokines, short stature, vitamin D deficiency

Introduction

Short stature in children is a persistent global health issue, with its prevalence continuing to rise across various regions.[1,2] According to the 2018 Joint Child Malnutrition Estimates, 22.2% or 150.8 million children under the age of five worldwide were affected by short stature in 2017.[3] This condition is often linked to both macronutrient and micronutrient deficiencies, with studies in developing countries like India and Pakistan identifying malnutrition and normal variance as primary causes.[4,5] In Indonesia, short stature remains prevalent, with the 2018 Riskesdas survey reporting a prevalence of 31.06% among children under five in West Java Province, reaching 40.7% in Bandung city.[6]

Malnutrition, macronutrient deficiencies (protein-energy malnutrition) and micronutrient deficiencies (such as vitamin and mineral deficiencies), is the leading cause of short stature and is associated with decreased leptin levels, which disrupt immune regulation.[7,8] In addition to nutritional deficiencies, hormonal imbalances involving growth hormone

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(GH) and insulin-like growth factor-1 (IGF-1) also contribute to impaired growth. Reduced IGF-1 levels can lead to thymus atrophy and altered T-cell differentiation, which compromises immune function and increases susceptibility to infections.[9,10]

Vitamin D deficiency is another factor that has been strongly associated with compromised immune function and an increased risk of infections in early childhood.[11] In particular, it has been linked to a higher incidence of upper respiratory tract infections (URTI) in children, with complications such as acute otitis media (AOM) being common. AOM affects a significant proportion of children, particularly those aged 6 months to 3 years, and has been associated with lower serum levels of 25-hydroxyvitamin D.[12,13] Studies have also shown that children with short stature often have vitamin D deficiency, further compounding their risk of infections such as AOM.[14,15]

The pathophysiology of AOM involves an inflammatory response within the middle ear, characterized by the accumulation of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and anti-inflammatory cytokines like interleukin-10 (IL-10). These cytokines play critical roles in the immune response and are key mediators in the inflammatory process of AOM. Without timely treatment, AOM can lead to serious health complications. Despite the well-established role of vitamin D and inflammatory cytokines in immune regulation, there is a lack of research examining their specific involvement in the development of AOM in children with short stature. This study aims to explore the relationship between serum levels of vitamin D, IL-6, TNF-α, and IL-10, and the occurrence of AOM in short-stature pediatric patients, providing insight into the potential role of these biomarkers in both the prevention and management of AOM in this vulnerable population.

Materials and Methods

This study employed a case-control design, involving children aged 24 to 59 months, who were categorized into four groups: short stature with AOM, short stature without AOM, normal stature with AOM, and normal stature without AOM. The study took place between July 2019 and February 2020 at 30 primary healthcare centers in Bandung Regency, using a multistage random sampling method. Ethical clearance was obtained from the Research Ethics Committee of the Faculty of Medicine, Padjadjaran University. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was collected from the parents or guardians of all participating children prior to their inclusion in the study.

Sampling and Height Measurements

Participants were selected based on specific inclusion and exclusion criteria. The inclusion criteria required children aged 24 to 59 months residing in Bandung Regency, while the exclusion criteria ruled out children who moved during the study period, as well as those with severe illnesses, congenital abnormalities, or conditions such as Down syndrome. A total of 800 children were selected from 1544 child records across 39 healthcare facilities in the Bandung region. The children's height was measured to categorize them as either having short stature or normal stature, following WHO standards. Children with a height-for-age Z-score < −2SD were classified as having short stature, while those between −2SD and 2SD were considered of normal height. After the informed consent process, interviews were conducted to collect data, which was verified using the Acute Otitis Media (AOM) questionnaire.

Measurement of 25(OH)D, IL-6, TNF-α, and IL-10

Blood samples were collected to measure serum levels of 25-hydroxyvitamin D (25(OH)D), IL-6, TNF-α, and IL-10. The 25(OH)D samples were obtained from the 2018–2019 ALG study and stored at −80°C prior to analysis. The levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method, with a wavelength of 405 nm and Euroimmune reagent kits. IL-6, TNF-α, and IL-10 concentrations were determined using the Sandwich ELISA technique with reagents from E lab science, kept at 18–25°C. The ELISA microplate was coated with human polyclonal antibodies, and results were measured using a spectrophotometer at 450 nm. All laboratory tests were conducted at the Clinical Pathology Laboratory of Hasan Sadikin Hospital in Bandung, with data analyzed as participant numbers increased.

AOM Questionnaire and Tympanometry Measurements

To assess the severity of AOM, a questionnaire was administered to parents or guardians, which included demographic information and specific questions related to AOM, adapted from the PERHATI-KL Otology Study Group Questionnaire. Tympanometry measurements were also conducted as an objective evaluation of middle ear function, measuring tympanic membrane mobility and impedance values in the auditory ossicles. The results were classified into five categories: A, B, As, Ad, and C, reflecting different degrees of middle ear pathology.

Statistical Data Analysis

The data were analyzed using descriptive, bivariate, and multivariate statistical methods. Descriptive statistics included frequencies and percentages for categorical variables and means and standard deviations for continuous variables. The Shapiro–Wilks test was applied to check for normal distribution. Group comparisons of 25(OH)D, IL-6, TNF-α, and IL-10 levels were analyzed using bivariate methods such as ANOVA, Kruskal–Wallis, Chi-Square, Fisher's Exact Test, and the Kolmogorov–Smirnov test. Multivariate analysis was conducted to assess the effect of multiple risk factors on AOM. Statistical significance was defined as a p-value ≤ 0.05, with values above 0.05 considered non-significant.

Results

The study involved 180 children, divided into four groups: 48 children with short stature and acute otitis media (AOM), 44 with short stature without AOM, 44 with normal stature and AOM, and 44 with normal stature without AOM. Basic characteristics, including age, gender, exclusive breastfeeding, exposure to cigarette smoke, parental education, occupation, and income, were compared across groups to evaluate potential confounding variables (Table 1). Categorical variables were reported as frequencies and proportions, while numerical variables were expressed as medians with interquartile ranges (IQR) due to non-normal data distribution. Chi-square analysis revealed a statistically significant difference between genders among study groups, with boys more likely to experience AOM than girls, particularly in the short stature group (p=0.006).

Vitamin D status was assessed by measuring serum 25(OH)D levels and categorized as sufficient (≥30 ng/mL), insufficient (21–29 ng/mL), or deficient (≤20 ng/mL) based on Endocrine Society guidelines (Table 2). It was found that

Table 1 Characteristic of Research Subjects

Characteristic	Group				p-value*	I vs II
	Short Stature with AOM (I) (n=48)	Short Stature without AOM (II) (n=44)	Normal Stature with aom (III) (n=44)	Normal Stature without AOM (IV) (n=44)		p-value*
Gender:					0,006	<0,001
Male	37	18	27	26		
Female	11	26	17	18		
Age (months):					0.071	0.450
24–35	11	14	20	11		
36–47	16	10	15	18		
48–59	21	20	9	15		
Exclusive breastfeeding (yes)	46 (95.8%)	40 (90.9%)	39 (88.6%)	41 (93.3%)	0.610	0.421
Exposure to cigarette smoke (yes)	37 (77.1%)	35 (79.5%)	34 (77.3%)	37 (84.1%)	0.832	0.775
Parental education:					0.522	0.387
Primary school	14	16	18	16		
Junior high school	15	18	13	13		

(*Continued*)

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Table 1 (Continued).

Characteristic	Group				p-value*	I vs II
	Short Stature with AOM (I) (n=48)	Short Stature without AOM (II) (n=44)	Normal Stature with aom (III) (n=44)	Normal Stature without AOM (IV) (n=44)		p-value*
Senior high school	17	9	12	13		
University	2	1	1	2		
Parental occupation:					0.854	0.329
Entrepreneur	5	6	3	6		
Private sector	7	3	6	6		
Labourer	27	31	31	27		
Civil servant	2	0	1	1		
Others	7	4	3	4		
Monthly income:					0.621	0.729
< 1.5 million IDR	30	30	31	28		
1.5–2.5 million IDR	15	13	9	11		
2.5–3.5 million IDR	2	1	4	3		
> 3.5 million IDR	1	0	0	2		

Note: *Chi-square analysis for categorical differences.

Table 2 Status of Vitamin D Among Research Group

Vitamin D Status	Group				p-value*
	Short Stature with AOM (I) (n=48)	Short Stature without AOM (II) (n=44)	Normal Stature with AOM (III) (n=44)	Normal Stature without AOM (IV) (n=44)	
Normal	1	3	6	13	<0.001
Insufficiency	14	26	29	22	
Deficiency	33	15	9	9	
Comparative analysis of Vitamin D Status among research group:					**p-value****
Short stature with AOM vs Short stature without AOM					0.001
Short stature with AOM vs Normal stature with AOM					<0.001
Short stature with AOM vs Normal stature without AOM					<0.001
Short stature without AOM vs Normal stature with AOM					0.105
Short stature without AOM vs Normal stature without AOM					0.011
Normal stature with AOM vs Normal stature without AOM					0.252

Notes: *Chi-square analysis for categorical differences; ***Chi-square analysis for trends.

Chi-square analysis demonstrated a significant association between group categorization and vitamin D status ($p < 0.001$). In the short-stature group with AOM, the majority of children had vitamin D deficiency (33 children), followed by insufficiency (14 children). In the short-stature group without AOM, vitamin D insufficiency was more common (26 children), followed by deficiency (15 children). There was a significant difference in vitamin D status between the short-stature group with AOM and the normal stature group with AOM, where the latter was dominated by insufficiency (29 children) and deficiency (9 children). Similarly, the vitamin D status in the short-stature group with AOM was significantly different from the normal stature group without AOM, which was dominated by insufficiency (22 children) and normal levels (13 children). Children with short stature and AOM exhibited a notable difference in vitamin D levels compared to those without AOM, with a higher prevalence of vitamin D deficiency. This suggests a strong correlation between vitamin D deficiency, short stature, and the presence of AOM. However, there was no significant difference in vitamin D status

between the short-stature group without AOM and the normal stature group with AOM (p = 0.105), both of which were dominated by insufficiency followed by deficiency. This suggests that either AOM, short stature, or both conditions may be linked to vitamin D insufficiency or deficiency. Lastly, there was a significant difference in vitamin D status between the normal stature group without AOM and the short stature group without AOM, but no significant difference between the normal stature group without AOM and the normal stature group with AOM (p = 0.252).

The IL-6 concentrations across the four groups are presented as medians and IQRs (Table 3). The short stature group with AOM showed the highest median IL-6 level of 12.15 pg/mL, reflecting a significant inflammatory response. In contrast, the short stature group without AOM had a lower median IL-6 of 8.50 pg/mL. The normal stature group with AOM had a median IL-6 of 1.18 pg/mL, while the normal stature group without AOM showed the lowest median IL-6 of 0.62 pg/mL. Statistical analysis indicated a significant difference in IL-6 levels among the four groups (p < 0.001), with each group displaying distinct IL-6 levels, underscoring the variation in inflammatory responses.

The IL-10 levels varied significantly across the groups (Table 4). The short stature group with AOM had the lowest median IL-10 level of 0.56 pg/mL, compared to the short stature group without AOM, which had a median IL-10 of 1.56 pg/mL. In the normal stature group with AOM, the median IL-10 level was substantially higher at

Table 3 Comparison of IL-6 Levels in Four Study Groups

IL-6 Levels (pg/mL)	Median (IQR)	p-value*
Groups		<0.001
Short stature with AOM (n = 48)	12.15 (10.17–17.02)	
Short stature without AOM (n = 44)	8.50 (6.70–12.14)	
Normal stature with AOM (n = 44)	1.18 (0.84–2.06)	
Normal stature without AOM (n = 44)	0.62 (0.19–1.18)	
Comparisons:		
Short stature with AOM vs Short stature without AOM		<0.001
Short stature with AOM vs Normal stature with AOM		<0.001
Short stature with AOM vs Normal stature without AOM		<0.001
Short stature without AOM vs Normal stature with AOM		<0.001
Short stature without AOM vs Normal stature without AOM		<0.001
Normal stature with AOM vs Normal stature without AOM		<0.001

Notes: *For two groups, the comparison was made using the Mann–Whitney test; for more than two groups, the comparison was made using the Kruskal–Wallis test.

Table 4 Comparison of IL-10 Levels in Four Study Groups

IL-10 Levels (pg/mL)	Median (IQR)	p-value*
Groups		<0.001
Short stature with AOM (n = 48)	0.56 (0.16–1.51)	
Short stature without AOM (n = 44)	1.56 (0.90–2.57)	
Normal stature with AOM (n = 44)	8.32 (2.55–14.80)	
Normal stature without AOM (n = 44)	14.69 (14.03–18.60)	
Comparisons:		
Short stature with AOM vs Short stature without AOM		<0.001
Short stature with AOM vs Normal stature with AOM		<0.001
Short stature with AOM vs Normal stature without AOM		<0.001
Short stature without AOM vs Normal stature with AOM		<0.001
Short stature without AOM vs Normal stature without AOM		<0.001
Normal stature with AOM vs Normal stature without AOM		<0.001

Notes: *For two groups, the comparison was made using the Mann–Whitney test; for more than two groups, the comparison was made using the Kruskal–Wallis test.

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8.32 pg/mL, while the normal stature group without AOM had the highest median IL-10 level of 14.69 pg/mL. Statistical analysis confirmed significant differences in IL-10 levels among all four groups ($p < 0.001$), suggesting an inverse relationship between IL-10 levels and the severity of inflammation in short stature children with AOM.

The TNF-α concentrations were highest in the short stature group with AOM, with a median level of 162.52 pg/mL, indicating a pronounced inflammatory response in this group (Table 5). The short stature group without AOM followed with a median TNF-α of 141.84 pg/mL. The normal stature group with AOM had a lower median TNF-α level of 122.36 pg/mL, while the normal stature group without AOM showed the lowest TNF-α level at 97.09 pg/mL. Despite these differences, statistical analysis showed no significant difference in TNF-α levels between the short stature groups with and without AOM ($p = 0.214$), suggesting that TNF-α levels alone may not serve as a reliable marker for AOM in children with short stature.

To analyze the multivariable relationship between 25(OH)D, IL-6, TNF-α, and IL-10 levels, a multivariate logistic regression analysis was performed. Since 25(OH)D, IL-6, TNF-α, and IL-10 levels are numerical data, the receiver operating characteristic (ROC) curve was first used to determine the cut-off values. The ROC curve analysis results are presented in Table 6.

Table 7 provides the results of the multivariable analysis, which included variables with highly significant p-values from the bivariate analysis: gender, 25(OH)D levels ≤18.9 ng/mL, IL-6 levels >9.42 pg/mL, and IL-10 levels ≤ 0.76 pg/mL. Based on the multivariate analysis shown in Table 8, it was found that short-stature male children have a 3.80 times higher risk of developing acute otitis media (AOM), short-stature children with 25(OH)D levels ≤18.9 ng/mL have a 5.49 times higher risk, those with IL-6 levels >9.42 pg/mL have a 67.58 times higher risk, and children with IL-10 levels ≤0.76 pg/mL have a 29.20 times higher risk of developing AOM.

Table 5 Comparison of TNF-α Levels in Four Study Groups

TNF-α Levels (pg/mL)	Median (IQR)	p-value*
Groups		<0.001
Short stature with AOM (n = 48)	162.52 (97.82–225.29)	
Short stature without AOM (n = 44)	141.84 (96.75–165.81)	
Normal stature with AOM (n = 44)	122.36 (86.38–158.79)	
Normal stature without AOM (n = 44)	97.09 (66.96–129.37)	
Comparisons:		
Short stature with AOM vs Short stature without AOM		0.214
Short stature with AOM vs Normal stature with AOM		0.030
Short stature with AOM vs Normal stature without AOM		<0.001
Short stature without AOM vs Normal stature with AOM		0.278
Short stature without AOM vs Normal stature without AOM		0.011
Normal stature with AOM vs Normal stature without AOM		<0.001

Notes: * For two groups, the comparison was made using the Mann–Whitney test; for more than two groups, the comparison was made using the Kruskal–Wallis test.

Table 6 Cut-off Values for 25(OH)D. IL-6. TNF-α. and IL-10 Levels in Predicting Acute Otitis Media in Short-Stature Children

Variable	Short-Stature Children with Acute AOM vs Short-Stature Children without AOM	
	cut off	AU ROC (CI 95%)
25(OH)D Levels	≤ 18.9 ng/mL	0.708 (0.604–0.798)
IL-6 Levels	>9.42 pg/mL	0.741 (0.640–0.827)
TNF-α Levels	>167.6 pg/mL	0.575 (0.468–0.678)
IL-10 Levels	≤ 0.76 pg/mL	0.762 (0.662–0.845)

Table 7 The Relationship Between Cut-off Values of 25(OH)D. IL-6. TNF-α. and IL-10 Levels with Acute Otitis Media in Short-Stature Children

Variable	cut off	Short Stature		p-value*	OR (CI 95%)
		AOM +	AOM -		
25(OH)D Levels	≤ 18.9 >18.9	30 (62.5) 18 (37.5)	11 (25.0) 33 (75.0)	<0.001	5.00 (2.04–12.28)
IL-6 Levels	>9.42 ≤9.42	43 (89.6) 5 (10.4)	17 (38.6) 27 (61.4)	<0.001	13.66 (4.51–41.33)
TNF-α Levels	>167.6 ≤167.6	24 (50.0) 24 (50.0)	10 (22.7) 34 (77.3)	0.007	3.40 (1.38–8.40)
IL-10 Levels	≤0.76 >0.76	28 (58.3) 20 (41.7)	7 (15.9) 37 (84.1)	<0.001	7.40 (2.75–19.93)

Note: *Chi-square correlative analysis.
Abbreviation: OR, Odds Ratio.

Table 8 Multivariable Analysis of Factors Associated with Acute Otitis Media in Short-Stature Children (Final Model)

Variable	Coefficient B	SE (B)	p-value	OR_{adj} (CI 95%)
Gender (Male)	1.335	0.686	0.052	3.80 (0.99–14.58)
25(OH)D Levels (≤18.9 ng/mL)	1.704	0.856	0.009	5.49 (1.52–19.89)
IL-6 Levels (>9.42 pg/mL)	4.213	1.127	<0.001	67.58 (7.42–615.54)
IL-10 Levels (≤ 0.76 pg/mL)	3.374	1.114	0.002	29.20 (3.29–258.968)

Note: Accuracy = 85.9%; R^2 (Nagelkerke) = 0.688; p-value for TNF-α = 0.116.
Abbreviations: AUC, Area under the curve; LS, Least squares; NE, Not estimable; IDR, Indonesian Rupiah.

Discussion

Vitamin D, a fat-soluble vitamin, has gained recognition for its extensive biological effects beyond calcium homeostasis and bone metabolism. It plays a key role in modulating both innate and adaptive immune responses, with vitamin D receptors (VDRs) present on various immune cells, including monocytes, macrophages, dendritic cells, and lymphocytes. The active form, 1,25-dihydroxyvitamin D3, enhances antimicrobial peptide production, such as cathelicidin and defensins, which are vital in the innate immune response against pathogens.[16] Moreover, it regulates the adaptive immune system, promoting regulatory T cells while suppressing pro-inflammatory cytokines like IL-17 and IFN-γ.[14,17] These immune-related effects are relevant to conditions like asthma, where vitamin D deficiency has been associated with exacerbations and reduced airway inflammation in children.[18,19]

The relationship between vitamin D and short stature has also been well-documented. Studies show that children with short stature tend to have a higher prevalence of vitamin D deficiency compared to those with normal height.[14,20–22] This deficiency impacts the growth plate, impairing linear growth through reduced chondrocyte proliferation and differentiation, as well as diminished expression of key growth factors like IGF-1 and FGF-21.[23,24] Furthermore, vitamin D deficiency has been shown to negatively affect the GH-IGF-1 axis, essential for bone growth and development.[25] Research supports the use of vitamin D supplementation to improve growth outcomes in children with short stature and deficiency.[26]

In our study, we found a significant association between vitamin D deficiency, elevated inflammatory markers (IL-6, IL-10, and TNF-α), and increased risk of acute otitis media (AOM) in children with short stature. Children with short stature and AOM exhibited higher levels of IL-6 and lower levels of IL-10 compared to other groups. Elevated IL-6 levels suggest an enhanced inflammatory response, contributing to AOM's persistence.[27,28] The decreased IL-10 levels indicate an impaired resolution of inflammation, prolonging infection susceptibility.[29]

While the role of TNF-α in AOM has been debated, our study found no significant difference in TNF-α levels between children with short stature with or without AOM, indicating it may not serve as a reliable biomarker in this context. This observation reflects the complexity of the inflammatory response, which involves multiple pathways and factors.[30]

We evaluated the diagnostic accuracy of vitamin D, IL-6, IL-10, and TNF-α levels in predicting AOM in children with short stature. IL-10 emerged as the most accurate biomarker, followed by IL-6 and vitamin D, while TNF-α showed the lowest predictive value. These findings suggest that vitamin D deficiency, combined with altered inflammatory marker levels, may serve as indicators for AOM risk in this population.

Based on multivariable analysis, the factors associated AOM in children with short stature include male sex, 25(OH)D levels ≤18.9 ng/mL, IL-6 levels >9.42 pg/mL, and IL-10 levels ≤0.76 pg/mL. Short-statured boys are at a fourfold increased risk of developing AOM compared to girls. The study findings reveal a notable gender disparity, with short-statured male children at a significantly higher risk of developing Acute Otitis Media (AOM) compared to their female counterparts. Several factors may explain this gender difference. Anatomical differences between boys and girls, particularly in the structure of the Eustachian tube, may contribute to the increased susceptibility of boys to Acute Otitis Media (AOM). In children, the Eustachian tube is shorter and more horizontal compared to adults, which can impair the drainage of the middle ear and make it more susceptible to infections like AOM. Furthermore, research indicates that the average length of the cartilaginous and bony parts of the Eustachian tube is smaller in women than in men, which may affect the function of the Eustachian tube and contribute to the increased risk of AOM in boys.[31–33] Additionally, hormonal differences could play a role. Estrogen, which is more prevalent in girls, has been shown to have protective effects on the immune system, potentially offering girls more resistance to infections like AOM. Moreover, immune system differences between genders could contribute to the disparity; females typically have stronger immune responses, which may confer protection against infections.[31–33] Environmental factors, such as increased exposure to risk factors like daycare attendance, passive smoke exposure, and respiratory infections, may also differ between genders and influence AOM susceptibility. Genetic predispositions specific to boys may further contribute to this heightened risk. The study's results are consistent with prior research that has found male sex to be a significant risk factor for otitis media, further emphasizing the importance of considering gender in the management and prevention of AOM in pediatric populations. Understanding these underlying factors can help pediatricians and healthcare professionals identify children at higher risk for AOM, especially those with short stature, vitamin D deficiency, and elevated inflammatory markers like IL-6 and IL-10, and develop targeted preventive strategies.

Short-statured children with 25(OH)D levels ≤18.9 pg/mL have a 5.49-fold increased risk of developing AOM compared to those with levels >18.9 pg/mL. There are no previous reports explicitly linking vitamin D levels to AOM in this context. However, various studies have examined the correlation between vitamin D and otitis media, suggesting that vitamin D deficiency is associated with an increased risk of otitis media. A systematic review and meta-analysis by indicated that lower vitamin D levels are linked to otitis media.[34] Furthermore, a systematic review and meta-analysis reported that patients with otitis media had lower serum vitamin D levels.[35] Children with IL-6 levels >9.42 pg/mL are at a 67.58-fold increased risk of developing AOM compared to those with IL-6 levels ≤9.42 pg/mL.[35] Although previous studies have not reported on this specific relationship, Serban et al (2021) demonstrated that elevated IL-6 concentrations correlate with otitis media in children, implicating IL-6 in the inflammatory response associated with otitis media.[36] Additionally, short-statured children with IL-10 levels ≤0.76 pg/mL are at a 29.30-fold increased risk of developing AOM compared to those with IL-10 levels >0.76 pg/mL, though there are no prior studies documenting this finding.[36]

The multivariable analysis yielded an R^2 (Nagelkerke) value of 0.688, indicating that 68.8% of AOM cases in short-statured children can be attributed to the four aforementioned factors: male sex, 25(OH)D levels, IL-6 levels, and IL-10 levels. The remaining 31.2% may be influenced by other unexamined factors in this study.

Overall, our results underscore the importance of vitamin D status in modulating immune responses and inflammatory processes in children with short stature and AOM. The findings also align with previous research linking vitamin D deficiency to both impaired growth and increased susceptibility to infections.[21,31–33] Short stature itself may reflect underlying endocrine dysfunctions, such as disturbances in the GH-IGF-1 axis, which has been linked to immune modulation. IGF-1 deficiency could impair mucosal immunity, leading to an increased risk of recurrent infections like AOM. Additionally, chronic low-grade

inflammation in short-stature children, as indicated by elevated TNF-α and IL-6 levels, may contribute to an exaggerated immune response, further predisposing them to infections. This immune imbalance, coupled with vitamin D deficiency, may explain the increased susceptibility to AOM in short-stature children. Furthermore, the observed male predominance in AOM cases could be attributed to sex-based differences in immune responses, where boys tend to exhibit higher pro-inflammatory cytokine activity and lower regulatory cytokine levels. Further studies are needed to clarify the role of vitamin D supplementation in preventing AOM and to validate the use of these biomarkers in clinical settings.

This study has several limitations. First, the relatively wide 95% confidence interval may be attributed to the small sample size, despite meeting the minimum required number of subjects. A smaller sample size can lead to greater variability in estimates, resulting in wider confidence intervals. While the wide confidence interval indicates the need for more data to obtain more precise parameter estimates, it still encompasses values with clinically significant implications, warranting further research with a larger cohort. Second, there may be confounding variables that could influence the independent variables (25(OH)D, IL-6, TNF-α, and IL-10) or the dependent variable (AOM), which could not be measured in this study. These confounders include sunlight exposure, nutritional intake, allergens, pollutants, and genetic factors. Long-term follow-up studies are needed to assess changes in 25(OH)D, IL-6, TNF-α, and IL-10 levels, as well as the progression of AOM, to clarify and validate the impact of these variables on AOM in children with short stature.

Conclusion

This study highlights the complex interplay between short stature, vitamin D deficiency, and inflammatory markers in pediatric patients, particularly concerning the risk of Acute Otitis Media (AOM). Our findings reveal that children with short stature and AOM exhibit significantly elevated levels of IL-6 and TNF-α, indicating a pronounced inflammatory response compared to their counterparts without AOM. The notably low levels of IL-10 in this group further suggest an impaired regulatory response to inflammation, which may contribute to the heightened susceptibility to AOM in these children.

The multivariate analysis underscores critical risk factors for developing AOM, including male gender and low serum levels of vitamin D, IL-6, and IL-10. Specifically, male children with vitamin D levels ≤18.9 ng/mL and IL-6 levels >9.42 pg/mL face markedly increased odds of experiencing AOM, which aligns with previous literature highlighting the influence of gender on the incidence of ear infections in pediatric populations.

These results advocate for a multifaceted approach to managing children with short stature. Addressing vitamin D deficiency and monitoring inflammatory markers could provide essential insights for predicting and preventing AOM in this vulnerable group. Additionally, the strong correlation between vitamin D status and inflammatory responses calls for further investigation into the therapeutic benefits of vitamin D supplementation in improving immune function and reducing the incidence of AOM.

Given the significant association between vitamin D deficiency and increased risk of AOM in children with short stature, we recommend regular screening for vitamin D levels in this population. Healthcare providers should consider implementing vitamin D supplementation strategies, alongside monitoring inflammatory markers, to enhance immune response and mitigate the risk of AOM. Future research should focus on the long-term effects of vitamin D supplementation on the incidence of AOM and overall health outcomes in children with short stature. Based on the findings, OME screening should also be considered for children with short stature, particularly males, who are at a higher risk of developing AOM. Studies show that male children have anatomical differences that predispose them to a higher incidence of ear infections, including OME. Given the established risk factors—such as vitamin D deficiency, inflammation, and male gender—it would be prudent to implement routine OME screening in these vulnerable children to enable early diagnosis and intervention. Early detection of OME can prevent long-term complications like hearing loss, which may affect speech development and academic performance.

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Disclosure

The authors report no conflicts of interest in this work.

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