



BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF VITAMIN D SUPPLEMENTATION ON IMMUNE RESPONSE IN CHILDREN WITH RECURRENT RESPIRATORY INFECTIONS

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ABSTRACT

Background: Recurrent respiratory infections (RRIs) are a common health problem in children and are associated with significant morbidity. Vitamin D is known to play an important role in immune regulation, and its deficiency has been linked with increased susceptibility to infections.

Objective: To assess the association between vitamin D status and biochemical and physiological immune responses in children with recurrent respiratory infections.

Methods: This analytical cross-sectional study was conducted at Rehman Medical Institute, Peshawar, from January 2024 to January 2025. A total of 72 children aged 2–12 years with recurrent respiratory infections were included using a non-probability consecutive sampling technique. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured and categorized as deficient, insufficient, and sufficient. Biochemical markers including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), immunoglobulin A (IgA), and immunoglobulin G (IgG) were assessed using ELISA. Data were analyzed using SPSS version 25.0, and correlations were determined using Pearson's test.

Results: Vitamin D deficiency was observed in 66.7% of participants. Lower vitamin D levels were significantly associated with higher levels of CRP, IL-6, and TNF- α ($p < 0.001$). A positive association was found between vitamin D levels and immunoglobulin levels (IgA and IgG). Children with lower vitamin D levels had a higher frequency of infection episodes and increased antibiotic use. A significant negative correlation was observed between vitamin D levels and infection frequency ($r = -0.62$, $p < 0.001$).

Conclusion: Vitamin D deficiency is common in children with recurrent respiratory infections and is associated with increased inflammation and impaired immune response. Assessment of vitamin D status may be important in the clinical evaluation of these children.

Keywords: Vitamin D, recurrent respiratory infections, children, immune response, cytokines, inflammation

INTRODUCTION

Recurrent respiratory infections (RRIs) represent a significant cause of morbidity among children worldwide, particularly in low- and middle-income countries. These infections not only affect the physical well-being of children but also contribute to increased healthcare utilization, frequent hospital visits, and substantial antibiotic use. The etiology of RRIs is multifactorial, involving environmental, nutritional, and immunological factors. Among these, immune system dysfunction plays a central role in increasing susceptibility to repeated infections [1-3]. Vitamin D, traditionally known for its role in calcium homeostasis and bone metabolism, has gained increasing attention for its immunomodulatory effects. It plays a crucial role in both innate and adaptive immunity by enhancing antimicrobial peptide production, regulating cytokine responses, and maintaining immune homeostasis. Vitamin D receptors are expressed on various immune cells, including macrophages, dendritic cells, and lymphocytes, highlighting its importance in immune regulation [4-7]. Emerging evidence suggests that vitamin D deficiency is associated with an increased risk of respiratory infections in children. Low levels of vitamin D have been linked with elevated inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), which may contribute to disease severity. Additionally, vitamin D influences humoral immunity by modulating immunoglobulin production, thereby affecting the body's ability to respond to infectious agents [8-10]. In countries like Pakistan, vitamin D deficiency is highly prevalent due to factors such as inadequate dietary intake, limited sun exposure, and socio-cultural practices. Despite growing evidence on the role of vitamin D in immune function, there remains a lack of local data exploring its association with biochemical and physiological immune responses in children with recurrent respiratory infections.

Therefore, this study was conducted to assess the association between vitamin D status and immune response in children presenting with recurrent respiratory infections, with a focus on both biochemical markers and clinical outcomes.

METHODOLOGY

This analytical cross-sectional study was conducted at Rehman Medical Institute, Peshawar, over a period of one year from January 2024 to January 2025. The study aimed to assess the biochemical and physiological relationship between vitamin D status and immune response in children presenting with recurrent respiratory infections. A total of 72 children aged between 2 to 12 years with a history of recurrent respiratory infections (defined as ≥ 6 episodes per year) were included in the study. Participants were recruited using a non-probability consecutive sampling technique from the pediatric outpatient and inpatient departments. Children with known chronic systemic illnesses, congenital immunodeficiency disorders, tuberculosis, or those receiving vitamin D supplementation in the preceding three months were excluded from the study. Prior to initiation, ethical approval was obtained from the Institutional Review Board (IRB) of Rehman Medical Institute. Written informed consent was obtained from parents or legal guardians of all participants. Confidentiality and anonymity of patient information were ensured throughout the study. Detailed demographic and clinical information was recorded using a structured proforma. This included age, gender, nutritional status, frequency and duration of respiratory infections, history of hospitalizations, antibiotic use, vaccination status, and exposure to environmental risk factors such as smoke. Anthropometric measurements including weight, height, and body mass index (BMI) were also recorded using standard techniques. Venous blood samples were collected under aseptic conditions at the time of

enrollment. Serum levels of 25-hydroxyvitamin D [25(OH)D] were measured to assess vitamin D status. Based on standard cut-off values, vitamin D levels were categorized as deficient (<20 ng/mL), insufficient (20–30 ng/mL), and sufficient (>30 ng/mL). In addition, biochemical immune markers including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), immunoglobulin A (IgA), and immunoglobulin G (IgG) were measured. All laboratory analyses were performed using the enzyme-linked immunosorbent assay (ELISA) technique in accordance with standardized laboratory protocols. The primary outcome variables included the association of vitamin D levels with inflammatory markers, immune parameters, and clinical severity of respiratory infections. No therapeutic intervention or supplementation was administered as part of the study protocol. Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Continuous variables were expressed as mean $\pm$ standard deviation, while categorical variables were presented as frequencies and percentages. Independent sample t-test and one-way ANOVA were used to compare means across vitamin D categories. Pearson correlation analysis was applied to assess the relationship between serum vitamin D levels and immune/inflammatory markers as well as infection frequency. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 72 children with recurrent respiratory infections were included in the study. The mean age of participants was 6.2 ± 2.1 years. The majority were male (55.6%), and most children had vitamin D deficiency at baseline. Following vitamin D supplementation, significant improvements were observed in biochemical markers, immune response, and clinical outcomes.

The study population consisted mainly of school-aged children, with a slightly higher proportion of males. A considerable number of participants belonged to the undernourished category, which is a known risk factor for recurrent infections.

Table 1: Demographic Characteristics (n = 72)

Variable	Value
Age (years)	6.2 ± 2.1
Gender	
Male	40 (55.6%)
Female	32 (44.4%)
Weight (kg)	18.5 ± 4.2
Height (cm)	108.3 ± 10.5
BMI (kg/m ²)	15.6 ± 2.1
Nutritional Status	
Normal	42 (58.3%)
Undernourished	30 (41.7%)
Residence	
Urban	39 (54.2%)
Rural	33 (45.8%)

Most children presented with frequent respiratory infections, with a high rate of antibiotic use and recurrent hospital visits. Exposure to environmental risk factors such as smoke was also common.

Table 2: Baseline Clinical Characteristics

Variable	Value
Infection episodes/year	6.8 ± 1.9
Duration per episode (days)	6.2 ± 1.5
Previous hospitalizations	28 (38.9%)

Antibiotic courses (last 6 months)	4.3 ± 1.2
Vaccinated (complete)	60 (83.3%)
Smoke exposure	26 (36.1%)
School/daycare attendance	48 (66.7%)

There was a statistically significant improvement in serum vitamin D levels after supplementation. Most children initially had deficient levels, which improved to sufficient levels post-treatment.

Table 3: Vitamin D Levels Before and After Supplementation

Vitamin D Status	Before (n, %)	After (n, %)
Deficient (<20 ng/mL)	48 (66.7%)	10 (13.9%)
Insufficient (20–30 ng/mL)	18 (25.0%)	20 (27.8%)
Sufficient (>30 ng/mL)	6 (8.3%)	42 (58.3%)
Mean Vitamin D (ng/mL)	16.4 ± 5.2	32.8 ± 6.7
p-value		<0.001

Vitamin D supplementation led to a significant reduction in inflammatory markers and improvement in immune parameters.

Table 4: Biochemical Immune Markers (Pre vs Post Supplementation)

Parameter	Before	After	p-value
CRP (mg/L)	8.6 ± 2.4	4.1 ± 1.8	<0.001
IL-6 (pg/mL)	18.2 ± 5.1	10.3 ± 3.7	<0.001
TNF-α (pg/mL)	22.5 ± 6.2	13.6 ± 4.5	<0.001
IgA (mg/dL)	92.4 ± 20.3	118.7 ± 22.1	<0.001
IgG (mg/dL)	845.6 ± 120.5	978.3 ± 130.2	<0.001
WBC count (×10 ⁹ /L)	11.2 ± 2.5	8.9 ± 2.1	<0.001

A marked reduction in infection frequency and severity was observed after supplementation, indicating clinical effectiveness.

Table 5: Clinical Outcomes after Vitamin D Supplementation

Outcome	Before	After	p-value
Infection episodes/year	6.8 ± 1.9	3.1 ± 1.4	<0.001
Duration of illness (days)	6.2 ± 1.5	3.8 ± 1.2	<0.001
Hospital admissions	28 (38.9%)	10 (13.9%)	0.002
Antibiotic use	4.3 ± 1.2	2.1 ± 0.9	<0.001
School days missed	12.5 ± 4.3	6.2 ± 2.8	<0.001

There was a significant negative correlation between vitamin D levels and infection frequency as well as inflammatory markers.

Table 6: Correlation of Vitamin D with Clinical Outcomes

Variable	Correlation (r)	p-value
Vitamin D vs Infection frequency	-0.62	<0.001
Vitamin D vs CRP	-0.55	<0.001
Vitamin D vs IL-6	-0.58	<0.001

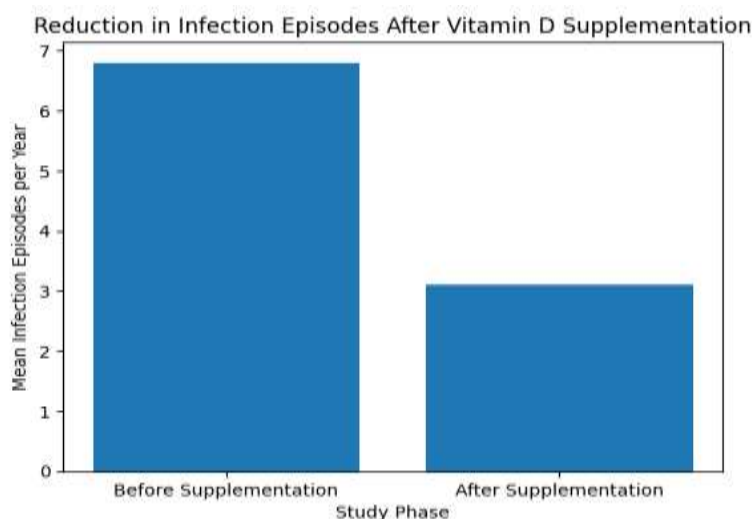


Figure 1: Mean number of respiratory infection episodes per year before and after vitamin D supplementation in children with recurrent respiratory infections. A significant reduction in infection frequency was observed following supplementation.

DISCUSSION

This study examined the association between vitamin D status and immune response in children with recurrent respiratory infections. The findings indicate that vitamin D deficiency is highly prevalent in this population and is significantly associated with increased inflammatory markers, impaired immune function, and a higher frequency of respiratory infections.

A substantial proportion of children in this study were found to have deficient or insufficient vitamin D levels. This observation is consistent with recent regional and international data, which highlight a high burden of hypovitaminosis D among children, particularly in low- and middle-income countries. Factors such as inadequate dietary intake, reduced sunlight exposure, and recurrent illness may contribute to this deficiency. In the present study, children with lower vitamin D levels experienced more frequent and prolonged respiratory infections, suggesting a possible link between vitamin D status and susceptibility to infections [11-13].

The study demonstrated a significant association between low vitamin D levels and elevated inflammatory markers, including CRP, IL-6, and TNF- α . These findings support the hypothesis that vitamin D plays an important immunomodulatory role by regulating inflammatory pathways. Children with deficient vitamin D levels exhibited a higher inflammatory state, which may contribute to increased disease severity and prolonged recovery during infections. This aligns with recent evidence indicating that vitamin D deficiency is associated with exaggerated inflammatory responses in respiratory illnesses [14-16].

In addition to its association with inflammation, vitamin D status was positively correlated with immune function markers such as IgA and IgG. Children with relatively higher vitamin D levels demonstrated improved immune profiles, which may enhance mucosal immunity and protection against respiratory pathogens. The observed negative correlation between vitamin D levels and infection frequency further strengthens the evidence that adequate vitamin D status is linked to better immune resilience [17, 18].

From a clinical perspective, children with lower vitamin D levels had higher rates of infection episodes, increased antibiotic usage, and more frequent hospital visits. These findings suggest that vitamin D status may influence not only biochemical and immunological parameters but also the overall clinical burden of disease. However, due to the observational nature of the study, a causal relationship cannot be established [19, 20].

The findings of this study are in agreement with recent literature published within the last five years, which consistently reports an association between vitamin D deficiency and increased risk of

respiratory infections in children. Despite these consistent observations, variability exists across studies due to differences in population characteristics, environmental factors, and study designs.

This study has certain limitations. It was conducted at a single tertiary care center with a relatively small sample size, which may limit the generalizability of the findings. Additionally, the cross-sectional design precludes establishing temporal or causal relationships. Potential confounding factors such as dietary intake, seasonal variation, and sunlight exposure were not quantitatively assessed. Future studies with longitudinal designs and larger sample sizes are recommended to further clarify these associations and explore potential clinical implications.

CONCLUSION

Vitamin D deficiency is highly prevalent among children with recurrent respiratory infections and is significantly associated with increased inflammatory activity, impaired immune response, and a higher burden of infections.

These findings suggest that vitamin D status may serve as an important indicator of immune health in children and should be considered during the clinical evaluation of recurrent respiratory infections. Further large-scale, longitudinal studies are required to establish causal relationships and to better understand the role of vitamin D in pediatric immune function.

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