ORIGINAL ARTICLE

Diagnostic Significance of Serum Vitamin D Levels in Pediatric Acute Respiratory Infections: A Comparative Study of Covid-19 and Other Acute Respiratory Infections

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ABSTRACT

Objective: The aim of the study was to evaluate the diagnostic significance of serum 25-hydroxyvitamin D [25(OH)D] levels in children with acute respiratory diseases, with particular focus on COVID-19 and other respiratory infections.

Study design: Observational study

Place and Duration of Study: This study was conducted throughout 2021 at the Educational-Therapeutic Clinic of Azerbaijan Medical University and Baku City Children's Infectious Diseases Hospital №7, Baku, Azerbaijan.

Material and Methods: The study involved 110 children aged 1 to 17 years. Of these, 20 were diagnosed with acute respiratory diseases (ARDs) based on clinical presentation, laboratory, and imaging findings; 75 children tested positive for SARS-CoV-2 RNA in nasopharyngeal swabs (COVID-19 patients); and 15 healthy children formed the control group. Vitamin D levels in all participants' serum were measured using the enzyme-linked immunosorbent assay (ELISA) method utilizing the Stat Fax 4700 analyzer and reagent kits (Germany).

Results: Children with COVID-19 (PCR-positive) demonstrated an average vitamin D concentration of 25.1 ng/ml (median 24.8 ng/ml) ($p<0.001$), while those with ARDs had an average of 20.8 ng/ml (median 21.0 ng/ml) ($p<0.001$). The interquartile range (Q_1-Q_3) for vitamin D levels in the ARDs group was 18.3-23.8 ng/mL, whereas the COVID-19 group had 20.6-29.2 ng/ml. The control group had an average vitamin D level of 46.4 ng/ml (median 48.4 ng/ml), with an interquartile range (Q_1-Q_3) of 39.5-55.8 ng/ml.

Conclusion: According to the comprehensive findings of this study, low levels of vitamin D in serum represent a significant contributing factor to the development and worsening of respiratory diseases in children.

Key Words: Children, Respiratory diseases, Vitamin D, Immune system, SARS-CoV-2, 25-hydroxyvitamin D, Acute respiratory infections

INTRODUCTION

It is well established that the leading cause of morbidity and mortality in the pediatric population is respiratory infections, and the role of certain factors in the development of these diseases continues to be actively investigated[1,2]

Recent studies have demonstrated that vitamin D plays a crucial role in the development of respiratory tract diseases in children. Low levels of vitamin D in serum not only contribute to the development of respiratory diseases (such as ARDs, COVID-19, and other viral infections), but are also associated with an increased frequency and severity of exacerbations of these diseases.[3,4] It has been found that the higher incidence of viral infections during winter months is significantly associated with a decrease in the synthesis of vitamin D.

The primary objective of this study was to comprehensively assess the diagnostic significance of vitamin D levels in children with acute respiratory diseases, including COVID-19 and other respiratory infections.

MATERIAL AND METHODS

The study was conducted at the Educational-Therapeutic Clinic of Azerbaijan Medical University and the Children's Infectious Diseases Hospital №.3 throughout 2021. For our comprehensive study, 110 children aged 1-17 years were carefully selected. Of these, 95 were patients with respiratory infections, and 15 were healthy children serving as controls. Among the patients, 57 were boys (51.8%) and 53 were girls (48.2%).

Patient selection and criteria: Based on the anamnesis, comprehensive clinical signs, laboratory, and instrumental results of the pediatric patients, 20 children were diagnosed with Acute Respiratory Infections, while 75 children were diagnosed with COVID-19, as indicated by a positive SARS-CoV-2 RNA test in pathological material obtained from the nasal and throat swabs. The patients exhibited a moderately severe course of disease. They were examined during the acute phase of the disease.

Laboratory examinations: Laboratory examinations included complete blood count, comprehensive biochemical tests, and measurement of vitamin D levels. For evaluating vitamin D in serum, the concentration of 25 (OH) D form was considered an adequate and reliable indicator. The level of 25 (OH) D in the blood was assessed based on the following internationally accepted criteria: Normal: 25 (OH) D levels between 30-100 ng/ml; Insufficiency: 20-29 ng/ml; Deficiency: 10-20 ng/ml; Severe deficiency: below 10 ng/ml.

The exclusion: Criteria were rickets, bronchial asthma, autoimmune diseases, cystic fibrosis, primary and acquired immunodeficiency, severe course, and an asymptomatic mild course of COVID-19 (PCR positive), and multisystem inflammatory syndrome (MIS-C).

Statistical analysis: The statistical analysis of the obtained data was performed using IBM Statistics SPSS-26 software, applying comprehensive methods such as variation analysis (U-Mann-Whitney, H-Kruskal-Wallis), discriminant analysis (Chi-square Pearson), analysis of variance (F-Fisher), correlation analysis (ρ-Spearman), and ROC analysis. The null hypothesis was rejected at $p<0.05$ values, ensuring statistical significance of the results.

Ethical considerations: The study protocol was approved by the Azerbaijan Medical University Ethics Committee (Protocol date: 25.05.2021, number: 2021-18). Written informed consent forms were received from each participant's parent or legal guardian prior to registration. The study was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

Clinical presentation: In COVID-19 cases, patients exhibited symptoms such as intoxication syndrome, digestive disturbances, loss of smell and taste, headache, muscle pain, auscultatory changes in the lungs, weakened respiration, wheezing, and notable loss of smell and taste. In cases of ARDs, symptoms included varying degrees of upper respiratory tract damage syndrome, catarrhal signs, and pronounced rhinitis. In both comparison groups, the common symptoms included fever and cough.

Vitamin D level analysis: In the study, the vitamin D levels of the examined patients were compared with the corresponding indicators of 15 healthy children.

The patients were grouped into three distinct categories based on the amount of vitamin D in their serum. According to results, 58 patients (61.1%) were found to have insufficiency, and 21

patients (22.1%) had deficiency. In 16 patients (16.8%), vitamin D levels were within the normal range. In the control group, 14 children (93.3%) had normal levels of vitamin D, while 1 child (6.7%) exhibited insufficiency ($p\chi^2 < 0.001$). The frequency distribution of vitamin D insufficiency is presented in **fig 1**. Thus, it was found that the levels in the main group were significantly lower compared to the control group **(table 1)**.

The vitamin D level in the patients was found to be 1.9 times lower, or 47.8%, compared to the control group, with an average of 24.2 ng/ml (structural indicator, median 23.2 ng/ml). In the control group, the average vitamin D level was 46.4 ng/ml (median 48.4 ng/ml), ($p<0.001$). In the control group, the interquartile range (Q_1 and Q_3) was 39.5-55.8, while in the patient group, this range decreased to 20.4-27.9.

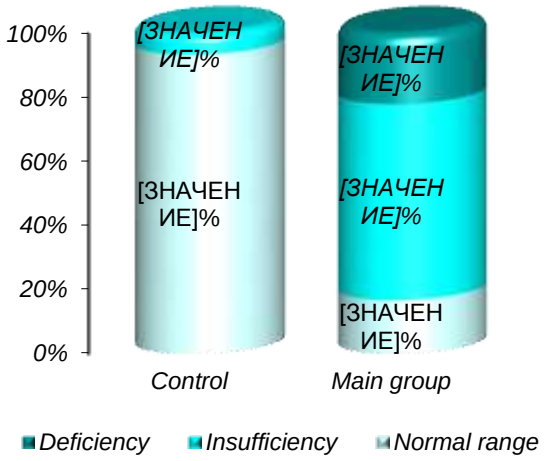

Fig 1: Vitamin D levels in children examined

TABLE 1: Vitamin D levels in children examined

Parameter	Group	N	M±m	Min±Max	Pu
Vitamin D(ng/ml)	Main	95	24.2±0.6	11.32±36.3	<0.001*
	Control	15	46.4±3.0	23.3±64.2	

Note: PU - The statistical significance of the group indicators was evaluated using the U-Mann-Whitney criterion. * - The null hypothesis is rejected.

Comparison Between ARDs and COVID-19: In the next phase of the study, the levels of vitamin D in patients with ARDs and COVID-19 (PCR positive) were compared with the corresponding indicators in the control group. The analysis of the obtained data revealed that the vitamin D levels were significantly lower in the ARDs and COVID-19 (PCR positive) groups compared to the control group, and the detailed results are presented in **table 2.**

TABLE 2: Vitamin D levels in patients with ARDs and COVID-19 (PCR positive)

Parameter	Group	N	M±m	Min Max	PH
Vitamin D (ng/ml)	COVID-19	75	25.1±0.6	14.2±36.3	0.001*
	ARDs	20	20.8±0.9	11.32±28.7	
	Control	15	46.4±3.0	23.36±64.2	

Note: PH - The statistical significance of the difference between group indicators was evaluated using the Kruskal-Wallis criterion. * - The null hypothesis is rejected.

Based on the comprehensive statistical analysis, the concentration of vitamin D shows that in children with COVID-19 (PCR positive), the levels were, on average, 25.1 ng/ml (structural indicator, median 24.8 ng/ml), which was significantly lower compared to the control group ($p<0.001$). In the ARDs group, the average vitamin D level was 20.8 ng/ml (structural indicator, median 21.0 ng/ml) ($p<0.001$). In the interquartile range, vitamin D levels in the ARDs group were between 18.3-23.8 ng/mL, while in the COVID-19 group, the levels ranged from 20.6-29.2 ng/ml. The control group had an average vitamin D level of 46.4 ng/ml (median 48.4 ng/ml), with an interquartile range (Q_1-Q_3) of 39.5-55.8 ng/ml **(fig 2).**

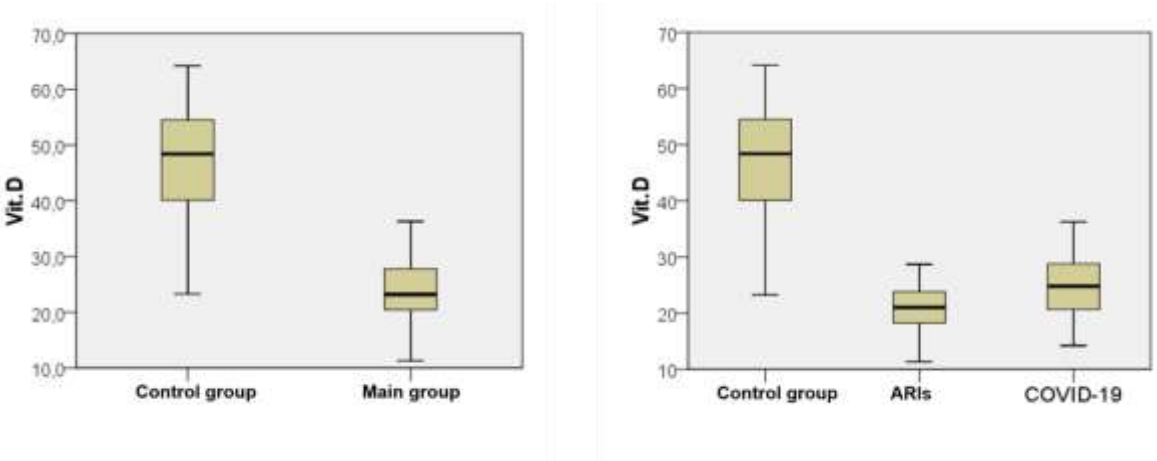

Fig 2: Average structural indicators of vitamin D levels in the serum of children in the comparison groups.

ROC Curve Analysis: In the study, an ROC curve was constructed to compare vitamin D levels in children with ARDs and COVID-19 (PCR positive) with the control group. Based on the ROC curve analysis, it was determined that the area under the ROC curve for vitamin D was 0.057±0.035. The 95% confidence interval (CI) was: upper bound 0.125, lower bound 0.000; p=0.000, indicating that vitamin D has high specificity and informativeness **(table 3 & fig 3).**

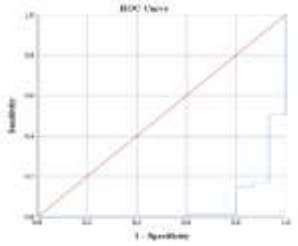

Fig 3: ROC-specific and sensitivity integral indicators of vitamin D in COVID-19 (PCR positive) and ARDs patients. (positive -95;negativ-15; missing-88)

TABLE 3: ROC-specific and sensitivity integral indicators of vitamin D in COVID-19 (PCR positive) and Acute Respiratory Infection patients

Test Result Variable(s)	Area	Standard error	Asymptotic Sig.	95% CI Lower Bound	95% CI Upper Bound
Vitamin D (ng/ml)	0.057	0.035	0.000	0.000	0,125

Age-Related Patterns: In the next phase of the study, the levels of vitamin D in children from different age groups were analyzed.

According to the amount of 25(OH)D in the serum in different age groups, the levels of vitamin D were as follows: In the <1 year age group, insufficiency was 40.9% and deficiency was 13.6%; in the 1-3 years age group, insufficiency was 53.8% and deficiency was 7.7%; and in the >3 years age group, insufficiency was 70.0% and deficiency was 28.3% (p<0.001) **fig 4**.

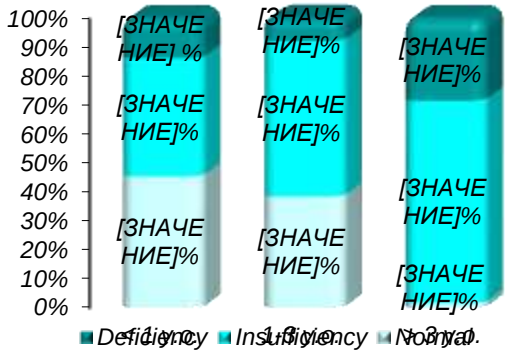

Fig 4. Vitamin D levels in different age groups.

The experiment demonstrates that there is a significant negative correlation between the vitamin D levels and the age of patients during COVID-19 and ARDs (ρ=-0.575, $p<0.001$ for COVID-19, and ρ=-0.489, $p=0.029$ for ARDs). Analysis of vitamin D levels across different age groups revealed that insufficiency and deficiency were primarily observed in older children.

Clinical correlations: In our study, a correlation between low vitamin D levels and the patient's clinical condition was observed. Specifically, a negative correlation was found between vitamin D levels and the clinical condition of the disease during COVID-19 and ARDs, with ρ=-0.227, $p=0.051$ for COVID-19 and ρ=-0.444, $p=0.050$ for ARDs.

A negative correlation was also observed between vitamin D levels and muscle pain (ρ=-0.412, $p<0.001$), shortness of breath (ρ=-0.366, $p<0.001$), loss of smell and taste (ρ=-0.338, $p=0.003$), and headaches (ρ=-0.259, $p=0.025$). These correlations could be related to an increased sensitivity of nociceptors (pain-sensitive receptors) and neurotransmitter receptors in response to VDR receptors during vitamin D deficiency.

DISCUSSION

This study represents a landmark achievement as the first comprehensive research conducted in Azerbaijan to simultaneously investigate vitamin D levels in children with both COVID-19 and acute respiratory diseases (ARDs). The findings provide crucial insights into the role of vitamin D in pediatric respiratory infections in our regional population.

Thus, in our study, vitamin D deficiency was observed in the vast majority of patients we examined. In our previous research, we had also observed low levels of vitamin D in children with COVID-19 (PCR positive),[5] which aligns with the current findings and confirms the consistency of this association.

Today, many researchers worldwide support the critical role of vitamin D deficiency in respiratory infections.[6-9] In a study by Barania et al. (2020) in Egypt, the serum 25 (OH)D level in children with respiratory infections was only 13.7 ng/ml, compared to 40.6 ng/ml in healthy controls. This value is even lower than our findings, reflecting a more severe deficiency.[10] Özdemir, Köksal, et al. reported that in the group with recurrent respiratory infections, the mean serum 25(OH)D level was 11.97 ± 4.04 ng/ml; in the chronic cough group, it was 13.76 ± 4.81 ng/ml; and in the control group, it was 31.91 ± 18.79 ng/ml.[11] In our study, the mean serum 25 (OH)D level in children with COVID-19 was 25.1 ng/ml, which was significantly lower than in the control group ($p < 0.001$). Our findings show interesting consistency with studies from other countries. For example, in Turkey, Bayramoğlu et al. (2021) reported a mean vitamin D level of 18.6 ng/ml in children with COVID-19 and linked deficiency cases to severe clinical courses of the disease.[12] In Greece, Kosmeri et al. (2022) reported a serum 25 (OH)D level of approximately 20 ng/ml in children, emphasizing the impact of this parameter on disease severity.[13] In Poland, Anita (2023) found a mean level of 19.8 ng/ml in children with COVID-19 and noted that deficiency increased the risk of complications.[14]

Zhang et al., in their study, demonstrated correlative links between vitamin D levels and infectious respiratory illnesses.[15] Jaybhaye, and others noted that vitamin D deficiency is observed in children who frequently suffer from respiratory infections.[16,17]

Gromova (2020), et al. analyzed the publications related to coronavirus and, based on the findings, concluded that vitamin D is crucial for mitigating the effects of the cytokine storm and compensating for chronic comorbidities.[18]

Vitamin D activates numerous factors (such as interferon-activating factors) against coronaviruses and other single-stranded RNA viruses, preventing their spread and replication, enhances the antiviral protection processes in epithelial cells of bronchi, stimulates the gene expression of antimicrobial peptides-cathelicidin and β-defensins, which play a crucial role in innate immunity, as well as the anti-inflammatory enzyme angiotensin-converting enzyme (ACE).[19,20] Moreover, vitamin D contributes to strengthening the synthesis of surfactants in the alveoli during respiratory illnesses, normalizes lung function, and thereby improves respiratory function. Esposito and Leli[21] highlighted that vitamin D deficiency can act as a risk factor for

bronchiolitis, recurrent otitis media and tuberculosis in children. The authors concluded that maintaining adequate concentration of vitamin D can be used as an affordable and effective method to prevent certain ARDs. In 2023, Anita reported that preventing vitamin D deficiency could enhance children's health potential and reduce the risk of many diseases, including severe forms of ARDs and COVID-19.[14]

Our findings remain important for the Azerbaijani pediatric population. Vitamin D deficiency was observed across all groups, with the most severe deficiency in ARDs compared to COVID-19, suggesting possible pathogen-specific differences in pathophysiological mechanisms. Overall, the findings support screening and supplementation strategies, but clinical recommendations should remain cautious until confirmed by further trials.

Limitations: This study has several limitations that must be acknowledged. The COVID-19 pandemic significantly impacted our recruitment capabilities. During the pandemic period, parents brought their children to the hospital only in moderate to severe cases, while the majority of children with mild acute respiratory infections received treatment at home, substantially reducing the number of patients available for recruitment. Additionally, quarantine measures and social distancing rules limited the ability to bring healthy children for examination, as parents were understandably reluctant to expose their healthy children to potential infection risk in medical facilities.

Further constraints included ethical considerations, as obtaining blood samples from healthy children solely for research purposes during a pandemic was ethically challenging and securing parental consent proved difficult. The overwhelming burden on healthcare workers treating COVID-19 patients also hindered additional patient recruitment for research purposes.

In addition, it should be noted that during the COVID-19 quarantine, potential confounding factors such as nutritional status, exposure to sunlight, and socioeconomic factors that could affect vitamin D levels were not considered.

Consequently, our study was limited to 110 children (95 patients and 15 healthy controls). We fully acknowledge that this small sample size reduces the statistical power of the study and limits the generalizability of the results. The cross-sectional design also prevents establishment of causal relationships between vitamin D deficiency and respiratory infections.

Despite these limitations, this study provides the first systematic data on vitamin D levels in pediatric respiratory infections in Azerbaijan and establishes a critical foundation for future larger-scale investigations. Further prospective studies with larger sample sizes and consideration of confounding variables are warranted to confirm these findings and establish optimal vitamin D supplementation strategies for preventing respiratory infections in children.

The severity of respiratory infections in this study was determined based on clinical assessment rather than standardized scoring systems. Moreover, detailed outcome measures such as hospitalization duration, intensive care admission, and long-term follow-up were not analyzed, which limits the ability to correlate vitamin D levels with clinical prognosis.

CONCLUSIONS

Based on the comprehensive results of this study, insufficient levels of vitamin D in the blood serum can be considered one of the most significant factors influencing the development and exacerbation of respiratory diseases in children. The study demonstrates that children with respiratory diseases had significantly lower vitamin D levels compared to healthy controls, with maintaining adequate vitamin D levels above 30 ng/ml potentially reducing both susceptibility to and severity of respiratory infections in the pediatric population.

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Authors' contribution

IYH: Proposed topic, basic study design, material and methods, manuscript.
AQH: Data collection, statistical analysis and interpretation of result etc.
FMM: Literature review & referencing and quality insuer.
IAG: Material & methods and manuscript writing.
All the authors have approved the final manuscript draft and accept the responsibility of research integrity.