Serum IL-6 and IL-18 Responses in Pediatric COVID-19: Role of Vitamin D Status in a Cohort from Azerbaijan

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Abbreviations:
COVID-19: Coronavirus Disease 2019
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2
IL: Interleukin
PCR: Polymerase Chain Reaction
WHO: World Health Organization
MIS-C: Multisystem Inflammatory Syndrome in Children
ELISA: Enzyme-Linked Immunosorbent Assay
CV: Coefficient of Variation
uANOVA: Univariate Analysis of Variance

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Abstract.

Background: This study investigated the effects of COVID-19 infection and vitamin D status on inflammatory cytokine levels in children during the pandemic. A cohort of 170 children aged 1–17 years with PCR-confirmed SARS-CoV-2 infection was enrolled. Serum levels of IL-6 and IL-18 were analyzed in relation to vitamin D status and COVID-19 diagnosis. Our analysis revealed that children

with vitamin D deficiency exhibited a trend toward increased levels of IL-6 and IL-18 compared to those with normal vitamin D levels; however, this association did not reach statistical significance. In contrast, COVID-19 infection was associated with significantly higher cytokine levels relative to healthy controls. These findings suggest a potential modulatory role of vitamin D in pediatric inflammatory responses to SARS-CoV-2, meriting further investigation through prospective studies.

Keywords: COVID-19; vitamin D; IL-6; IL-18.

1. Introduction

COVID-19, caused by the novel SARS-CoV-2 virus, emerged in Wuhan, China, in late 2019 and rapidly evolved into a global pandemic [1–3]. The clinical spectrum of the disease ranges from mild respiratory symptoms to severe pneumonia and acute respiratory distress syndrome (ARDS). A notable feature of the pandemic has been the generally milder or asymptomatic presentation observed in pediatric patients compared to adults, a phenomenon potentially attributable to differences in immune system maturation.

A critical pathogenic mechanism underpinning severe COVID-19 is the "cytokine storm," characterized by an excessive and dysregulated release of pro-inflammatory cytokines, including interleukin-6 (IL-6) and interleukin-18 (IL-18). This cascade can lead to uncontrolled systemic inflammation and multi-organ failure. As key mediators of immune regulation, cytokines are acutely elevated in response to viral replication in the upper respiratory mucosa. Despite growing evidence, the precise mechanisms governing cytokine dynamics in children remain incompletely elucidated.

Vitamin D has garnered significant interest for its immunomodulatory properties, influencing both innate and adaptive immune responses. Its active form, 1,25-dihydroxyvitamin D, enhances alveolar surfactant production, attenuates the expression of pro-inflammatory cytokines, and modulates the renin-angiotensin system in lung tissue [4–8]. The interplay between COVID-19, vitamin D status, and the ensuing inflammatory response in the pediatric population warrants further exploration. This study represents the first investigation of this relationship in children from Azerbaijan. The primary objective was to evaluate the effects of SARS-CoV-2 infection and vitamin D deficiency on serum levels of IL-6 and IL-18 in children with PCR-confirmed COVID-19.

2. Materials and Methods

2.1. Study Design and Participants

This study was conducted at the Children's Infectious Diseases Hospital in Baku, Azerbaijan, during 2022–2023, a period coinciding with ongoing pandemic lockdown measures. A total of 170 children (92 boys, 78 girls) aged 1–17 years were enrolled. All participants had PCR-confirmed SARS-CoV-2 infection and were clinically classified as having moderate (121 patients; 71.2%) or severe (49 patients; 28.8%) COVID-19. The standard clinical assessment for all patients included a detailed anamnesis, epidemiological history, physical examination, comprehensive laboratory testing, cytokine profiling, and radiological evaluation of the lungs, which typically revealed infiltrative shadows of varying sizes. **Patient clinical and laboratory data were accessed for research purposes between 15/01/2022 and 28/12/2023.**

The study protocol was reviewed and approved by the local Institutional Review Board, and informed consent was obtained from the parents or legal guardians of all participants. Children with rickets, bronchial asthma, autoimmune diseases, cystic fibrosis, primary or acquired immunodeficiency, other

chronic diseases, mild or asymptomatic COVID-19, and multisystem inflammatory syndrome in children (MIS-C) were excluded from the study.

2.2. Laboratory Analysis

Serum cytokine and vitamin D levels were measured using commercially available kits and a widely recognized analytical platform. Specifically, IL-6 and IL-18 concentrations were quantified using the Human IL-6 and Human IL-18 ELISA Kits (Invitrogen, USA), respectively. The serum concentration of 25-hydroxyvitamin D [25(OH)D] was determined via an electrochemiluminescence assay on a Roche Cobas e411 analyzer (Switzerland).

Vitamin D status was classified based on serum 25(OH)D concentrations as follows: normal (30–100 ng/mL), insufficiency (20–29 ng/mL), deficiency (10–20 ng/mL), and severe deficiency (<10 ng/mL) [9].

2.3. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 26. Given the non-normal distribution of some variables, non-parametric tests were employed, including the Mann-Whitney U test and the Kruskal-Wallis H test. Correlations were assessed using Pearson's correlation coefficient. To evaluate the effects of multiple factors simultaneously, univariate analysis of variance (uANOVA) was conducted. A p-value of less than 0.05 was considered statistically significant. A post-hoc power analysis was performed, confirming sufficient statistical power for the main model ($F(1,35)=7.54$, $p=0.009$, partial $\eta^2=0.177$, Cohen's $f=0.464$).

3. Results

3.1. Demographic and Clinical Characteristics

The majority of the patient cohort were urban residents (143 patients; 81.1%). The most frequently reported clinical symptoms included fever (155 patients; 91.2%), cough (123 patients; 72.4%), and muscle hypotonia (78 patients; 46.7%). Other noted symptoms were headache (27; 15.9%), muscle pain (23; 13.6%), cyanosis (22; 13.2%), and loss of smell or taste (13; 7.6%). Cytokine levels (IL-6 and IL-18) were analyzed in a subset of 75 patients, who were subsequently grouped by both vitamin D status and COVID-19 diagnosis for comparative analysis, as outlined in Table 1.

Table 1. Grouping of Children Based on Serum Vitamin D Levels and COVID-19 Diagnosis.

Group	Category	N
I	Vitamin D Normal	16
	Vitamin D Insufficiency	47
	Vitamin D Deficiency	12
II	Control	15
	COVID-19	75

3.2. Cytokine Levels by Vitamin D Status

The levels of IL-6 and IL-18 stratified by vitamin D status are presented in Table 2. A trend was observed wherein children with vitamin D deficiency had higher mean levels of both IL-6 (3.925

pg/mL) and IL-18 (461.917 pg/mL) compared to those with normal vitamin D levels (IL-6: 3.242 pg/mL; IL-18: 349.178 pg/mL) or insufficiency (IL-6: 2.694 pg/mL; IL-18: 352.146 pg/mL). However, these differences across the vitamin D status groups were not statistically significant.

Table 2. Levels of IL-6 and IL-18 According to Vitamin D Status.

Vitamin D Status	Cytokine	Mean (pg/mL)	Std. Error	95% CI
Normal	IL-6	3.242	0.539	2.170–4.315
	IL-18	349.178	30.391	288.752–409.603
Insufficiency	IL-6	2.694	1.489	0.000–5.654
	IL-18	352.146	83.923	185.285–519.007
Deficiency	IL-6	3.925	0.851	2.233–5.617
	IL-18	461.917	47.945	366.588–557.245

3.3. Cytokine Levels by COVID-19 Status

As shown in Table 3, a clear and statistically significant difference in cytokine levels was observed based on COVID-19 status. Children with PCR-confirmed COVID-19 exhibited markedly higher mean levels of IL-6 (4.156 pg/mL) and IL-18 (442.624 pg/mL) compared to the healthy control group (IL-6: 1.664 pg/mL; IL-18: 268.346 pg/mL).

Table 3. Levels of IL-6 and IL-18 According to COVID-19 Status.

COVID-19 Status	Cytokine	Mean (pg/mL)	Std. Error	95% CI
Control	IL-6	1.664	1.525	0.000–4.697
	IL-18	268.346	85.959	97.438–439.255
COVID-19	IL-6	4.156	0.402	3.358–4.955
	IL-18	442.624	22.632	397.626–487.621

3.4. ANOVA Analysis

The results of the univariate ANOVA (uANOVA) are summarized in Table 4. The analysis examined the influence of Group I (Vitamin D status), Group II (COVID-19 status), and their interaction on cytokine levels. The corrected model for IL-18 was statistically significant ($p=0.001$). The intercept was significant for both cytokines, reflecting the overall model fit. While the main effect of COVID-19 status (Group I) showed a non-significant trend for both IL-6 ($p=0.103$) and IL-18 ($p=0.069$), the main effect of vitamin D status (Group II) and the interaction term were not statistically significant. A post-hoc power analysis was performed based on the ANOVA results obtained for the variable *Vitamin D level* between the two age groups. According to the SPSS output, the ANOVA test yielded: $F(1, 35) = 7.54$; $p=0.009$. Partial $\eta^2=0.177$ Cohen's $f=0.464$.

Therefore, the analysis confirms that the obtained result had an adequate statistical power to detect the observed effect size at the 5% significance level.

Table 4. u-ANOVA Results for IL-6 and IL-18.

Source	Cytokine	Type III SS	df	Mean Square	F	Sig. (p)
Corrected Model	IL-6	84.875	4	21.219	2.443	0.053
	IL-18	552,058.696	4	138,014.674	5.003	**0.001**
Intercept	IL-6	141.522	1	141.522	16.294	**<0.001**
	IL-18	2,109,940.011	1	2,109,940.011	76.488	**<0.001**
Group I (COVID-19 Status)	IL-6	23.542	1	23.542	2.710	0.103
	IL-18	93,847.306	2	46,923.653	1.701	0.190
Group II (Vit. D Status)	IL-6	2.073	2	1.036	0.119	0.888
	IL-18	8,031.541	1	8,031.541	0.291	0.591
Group I × Group II	IL-6	1.332	1	1.332	0.153	0.696
	IL-18	22,796.123	1	22,796.123	0.826	0.366

Note: Group I refers to COVID-19 Status (Control, COVID-19). Group II refers to Vitamin D Status (Normal, Insufficient, Deficient).

3.5. Comparison with International Studies

A comparison of our findings with other international pediatric studies is presented in Table 5. Our cohort from Azerbaijan demonstrated a high prevalence of vitamin D deficiency (66.7% when combining deficiency and insufficiency). Notably, the mean IL-6 level observed in our study (4.16 pg/mL) was lower than values reported in studies from Italy, Egypt, and Turkey, which may reflect differences in disease severity, timing of sample collection, or population-specific factors.

Table 5. Comparison of IL-6 and IL-18 Levels with International Pediatric Studies.

Study	Country	N	IL-6 (pg/mL)	IL-18 (pg/mL)	Vit D Deficiency	p-value
Present Study	Azerbaijan	75	4.16	442.6	66.7%	0.069–0.103
Curatola 2021	Italy	62	23.7	NR	45%	<0.01
Shafiek 2021	Egypt	89	15.8	NR	58%	<0.001
Kamil 2020	Turkey	85	12.3	NR	71%	<0.001
Wang 2024*	15 countries	2,143	8.2–31.4	Limited	12–64%	Variable
NR = Not reported; *Meta-analysis.						

As a result, the disease of COVID-19 in children, as well as the level of vitamin D, at the same time, both indicators were evaluated as factors that play a role in the change of the IL-6,IL-18 indicator (Figure 1,2, 3,4).

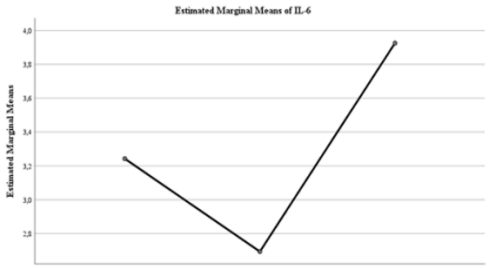

Figure 1. Variability of IL-6 levels by Vitamin D status.

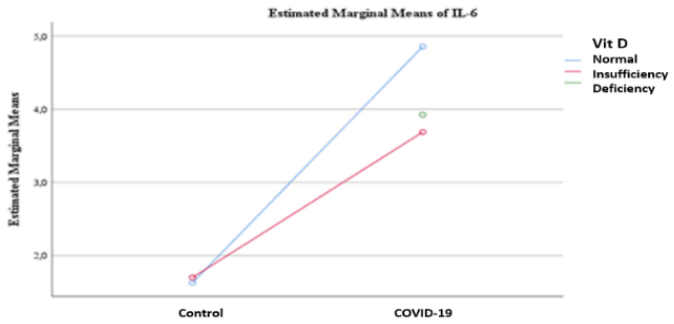

Figure 2. Variability of IL-6 levels by COVID-19 status.

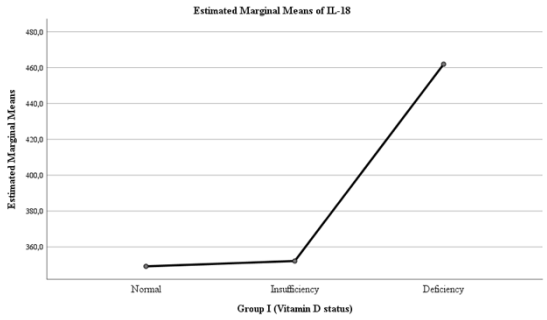

Figure 3. Variability of IL-18 levels by Vitamin D status.

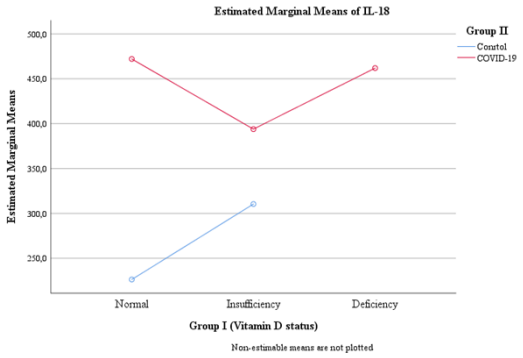

Figure 4. Variability of IL-18 levels by COVID-19 status.

4. Discussion

This study evaluated serum levels of the pro-inflammatory cytokines IL-6 and IL-18 in pediatric COVID-19 patients in relation to their vitamin D status. Our findings confirm that SARS-CoV-2 infection in children is associated with a significant elevation of these cytokines compared to healthy controls, aligning with the established pathophysiology of COVID-19 involving immune dysregulation and the cytokine storm phenomenon [10-16].

The central focus of our investigation was the potential modulatory role of vitamin D. We observed a consistent trend wherein children with vitamin D deficiency exhibited higher mean levels of both IL-6 and IL-18 compared to their counterparts with sufficient levels. This trend is biologically plausible, given vitamin D's known role in suppressing pro-inflammatory pathways and promoting immune homeostasis. However, this association did not achieve statistical significance in our cohort. This lack of significance may be attributable to several factors, including the relatively small sample size in the vitamin D deficient subgroup (n=12), which limits statistical power. Furthermore, the exclusive inclusion of children with moderate to severe disease may have reduced the variability needed to detect a moderating effect of vitamin D, as the inflammatory response in these patients is already pronounced.

When contextualized within the international literature (Table 5), our cohort exhibited a high prevalence of vitamin D deficiency, yet mean IL-6 levels were notably lower than those reported in other studies. This discrepancy could be influenced by ethnic and genetic factors, differences in predominant SARS-CoV-2 variants, or variations in clinical management. The cytokine storm, driven by IL-6 and IL-18, can cause significant damage to the alveolar epithelium, impair gas exchange, and contribute to the development of ARDS. The trend we observed suggests that vitamin D deficiency might exacerbate this inflammatory cascade, even if the effect is modest. Therefore, screening for vitamin D status in pediatric COVID-19 patients could still be valuable for identifying children who might be at a marginally increased risk of a heightened inflammatory state [17-20].

5. Conclusion

In summary, this study provides clear evidence that COVID-19 in children is associated with significantly elevated levels of the pro-inflammatory cytokines IL-6 and IL-18. While vitamin D deficiency was associated with a non-significant trend toward further increases in these cytokines, the potential for vitamin D to modulate the inflammatory response in pediatric COVID-19 should not be dismissed. The trends observed here highlight the need for more extensive, prospective studies to clarify this relationship. Future research, particularly interventional trials assessing the impact of vitamin D supplementation on cytokine profiles and clinical outcomes in pediatric COVID-19, is essential to determine whether a causal and therapeutically relevant relationship exists.

6. Study Limitations

This study has several limitations that should be considered when interpreting the results. Firstly, patient recruitment was challenged by strict quarantine measures and parental hesitancy, constraining the overall sample size. Secondly, the study was restricted to children with moderate and severe COVID-19; the inclusion of mild or asymptomatic cases could have provided a more comprehensive understanding of the cytokine response across the disease spectrum. Thirdly, the sample size, particularly for the subgroup with vitamin D deficiency, was limited, reducing the statistical power to detect potentially small but clinically meaningful effects. Finally, widespread lockdowns and associated lifestyle changes during the pandemic may have influenced vitamin D levels in the general population, potentially introducing a confounding variable that was not fully accounted for.

Author Contributions: Conceptualization, IH, AH; Data curation, FM, TT; Formal analysis, IG, AA; Investigation, FM, TT; Methodology, AH, NS; Project administration, IH; Resources, IH ; Software, IG; Supervision, IH, AH; Validation, AH, NS; Visualization, IG, AA; Writing–original draft, IH; Writing–review & editing, IH, AH, NS.

Institutional Review Board Statement: This study was conducted in accordance with the principles of the Declaration of Helsinki and complied with all relevant national guidelines and institutional

policies regarding research involving human participants. The study protocol was approved by the Local Ethics Committee of Azerbaijan Medical University (Approval No. 18; 2022).

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