10.4274/jcrpe.galenos.2023.2022-10-1

Research article

Can Serum 25 hydroxy Vitamin D Levels Predict the Severity of Multisystem Inflammatory Syndrome in Children and COVID-19?

Ekemen Keles Y et al. Vitamin D and Multisystem Inflammatory Syndrome

[1]Yildiz Ekemen Keles, MD*, [2]Dilek Yilmaz, MD, Prof, [1]Selin Tasar, MD [1]Gulnihan Ustundag, MD, [1]Aslihan Sahin, MD, [1]Aysegul Elvan Tuz, MD, [1]Aslihan Arslan Maden, MD, [1]Ahu Kara Aksay, MD, Asoc. Prof,[3] Ayfer Colak, MD, Asoc. Prof, [1]Eda Karadag Oncel, MD Asoc. Prof.
[1]Health Sciences University Tepecik Training and Research Hospital, Department of Pediatric Infectious Disease, Izmir, Turkey
[2]Izmir Katip Celebi University, Izmir, Department of Pediatric Infectious Disease, Izmir, Turkey
[3]Health Sciences University Tepecik Training and Research Hospital, Medical *Biochemistry Department*, Izmir, Turkey

What is already known on this topic?
Serum vitamin D levels are lower in patients with COVID-19 and MISC.

What this study adds?"
The severity of MISC is associated with low serum vitamin D levels.
There was a moderate correlation between the number of affected organ systems and serum 25 (OH) vitamin D levels.
The MISC patients who required intensive care unit stay had considerably lower vitamin D levels than those who did not.

Abstract
Objective: To determine the clinical significance of serum 25 hydroxy (OH) vitamin D levels in pediatric patients with multisystem inflammatory syndrome **(**MIS-C) and compare the vitamin D levels of these patients with those patients with COVID-19 and healthy controls.
Methods: This study was designed for pediatric patients who were aged 1 month to 18 years between July 14 and December 25, 2021. Fifty-one patients with MIS-C, 57 who were hospitalized with COVID-19, and 60 controls were enrolled in the study. Vitamin D insufficiency was defined as a serum 25 OH vitamin D level of less than 20 ng/mL.
Results: The median serum 25 (OH) vitamin D was 14.6 ng/mL in patients with MIS-C, 16 ng/mL in patients with COVID-19, and 21.1 ng/mL in the control group ($p<0.001$). Vitamin D insufficiency was present in 74.5% (n=38) of patients with MIS-C, 66.7% (n=38) of patients with COVID-19, and 41.7% (n=25) of the controls (p=0.001). The percentage of four or more affected organ systems was 39.2% in patients with MIS-C. The correlation between the number of affected organ systems and serum 25 (OH) vitamin D levels was evaluated in patients with MIS-C and there was a moderate negative correlation (r= -0.310; p=0.027). A weak negative correlation was found between the severity of COVID-19 and serum 25 (OH) vitamin D (r= -0.320, p=0.015).
Conclusion: It was found that vitamin D levels were insufficient in both groups and correlated with the number of affected organ systems of MIS-C and the severity of COVID-19.
Keywords: Vitamin D, COVID-19, MIS-C, Children

Yildiz Ekemen-Keles, MD, Health Sciences University Tepecik Training and Research Hospital, Department of Pediatric Infectious Disease, Izmir, Turkey
kutupylz@hotmail.com
90 544 774 98 26
0000-0002-6122-1726
11.10.2022
04.02.2023

Published: 16.02.2023

Introduction
The 2019 coronavirus disease pandemic (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has spread rapidly worldwide. While the nature of this disease is gradually being discovered, it has been observed that the clinical course is milder in children compared with adults (1). Nevertheless, recent evidence has shown that children may develop signs of multiorgan failure several weeks after primary infection, manifesting in cardiovascular dysfunction leading to life-threatening shock and even requiring a stay in the intensive care unit (ICU) due to the systemic inflammatory response (2). This novel syndrome was later termed multisystem inflammatory syndrome in children (MIS-C) (3, 4). Nevertheless, a postinfectious process is thought to be caused by non-neutralizing antibodies through antibody-dependent amplification, causing immune system dysregulation by SARS-CoV-2 with a racial genetic predisposition (5, 6).
Vitamin D is well-known for its role in regulating calcium and phosphorus metabolism. More recently, the role of vitamin D in non-skeletal functions, including inflammation and immune regulation, has also been investigated (7). One of the mechanistic

effects of vitamin D on immune function is the vitamin D receptor, which is expressed in most cell types and can influence genomic and nongenomic pathways related to the immune system (8). Vitamin D can induce monocyte differentiation into macrophages, increase the activity of lysosomal enzymes in macrophages, and facilitate cytotoxic activity by increasing the rate of phagocytosis (9). Many studies contain evidence that vitamin D reduces the risk of viral infection by suppressing the release of inflammatory cytokines derived from the adaptive immune system, particularly interleukin-2 and interferon-gamma (10, 11) Vitamin D has been reported to inhibit inflammatory processes by stimulating T-regulatory cells and increasing cellular immunity (10, 11). Vitamin D is also known to exert direct antibacterial and antiviral effects via cathelicidin. Cathelicidin is an antimicrobial peptide that promotes the induction of reactive oxygen radical synthesis, which has direct microbicidal effects and elicits immunomodulatory responses to pathogen-associated stimuli by recruiting neutrophils, monocytes, and T cells to microbial invasion sites (12, 13). The effect of vitamin D in MIS-C is thought to be due to its well-established role in modulating adaptive and innate immunity, including regulation of inflammatory cytokine release (5, 6).

There are many studies on vitamin D deficiency in children with various infectious diseases (14, 15). However, there are insufficient studies on vitamin D status in children with MISC. This study aimed to determine the clinical significance of serum 25 (OH) vitamin D levels in pediatric patients with MIS-C and to compare 25 (OH) vitamin D levels in patients hospitalized for COVID-19 and healthy controls.

Material and Methods

Study design

This prospective observational study was designed for pediatric patients who were aged 1 month to 18 between July 14th and December 25th, 2021. Hospitalized patients who met the diagnostic criteria for MIS-C were enrolled in the study. During the study period, hospitalized patients with a diagnosis of COVID-19 confirmed by a positive reverse transcriptase-polymerase chain reaction (RT-PCR) were included in the study. Healthy volunteers who were admitted to general pediatric polyclinics were defined as the control group and serum samples were taken at similar months to the patient group. The control group consisted of the 50th patient out of roughly 3000 applicants to pediatric outpatient clinics, as well as patients who were multiples of that patient.

Patient demographics, underlying disease, medication history, symptoms, laboratory results, system involvement, and outcomes were recorded in the medical records by completing the concept form. Clinical and laboratory parameters (lymphocyte count, neutrophil count, blood pressure, respiratory rate, and heart rate) were recorded as age-specific normal ranges. The need for ICU care due to inotropic support or fluid resuscitation, the need for invasive/non-invasive mechanical ventilation, or extracorporeal membrane oxygenation were assessed. Treatment modalities were recorded. The case definition of MIS-C was used as defined by the Centers for Disease Control and Prevention and the World Health Organization (3, 4). Severe MISC disease was classified as necessitating intensive care due to cardiovascular instability, the necessity for non-invasive or invasive mechanical ventilation, and/or a diminishing Glasgow coma scale. We used the World Health Organization definition criteria to represent the clinical stages of COVID-19 in children (16). We divided patients into four groups according to the clinical severity of COVID-19: asymptomatic, mild, moderate, severe-critical.

Cut-off values for serum 25 (OH) vitamin D have been previously announced with global consensus recommendations from pediatric endocrinologists: Vitamin D sufficiency is defined as a serum 25 (OH) vitamin D level of at least 20 ng/mL (50 nmol/l), whereas insufficiency is defined as 12 to 20 ng/mL (range, 30-50 nmol/L) and deficiency is less than 12 ng/mL (30 nmol/L) (17). Serum 25 (OH) vitamin D levels were measured during the first 3 days after hospitalization.

Patients who had taken vitamin supplements, who had bone metabolism disorders, and who did not want to participate in the study were excluded. Written informed consent was obtained from the patients and their parents. Ethical committee approval was obtained from Health Sciences University Izmir Tepecik Training and Research Hospital.

RT-PCR assay

Combined nasopharyngeal and oropharyngeal swab specimens were collected from children with suspected COVID-19 and sent to the medical microbiology laboratory. SARS-CoV-2 was detected using RT-PCR (Bio-Speedy SARS CoV-2 double Gene RT-qPCR Kit). Specifically, two target genes, including open reading frame 1ab (ORF1ab) and nucleocapsid protein (N), were tested during the RT-PCR assay.

Vitamin D assay

Blood samples were placed in gel-containing tubes with a clot activator (BD Vacutainer SST II Advance, USA) and centrifuged at 1500 *g* for 10 minutes to separate serum from clot. Serum 25 (OH) vitamin D was measured by chemiluminescence immunoassay on an Advia Centaur XP analyzer (Siemens Healthineers, Erlangen, Germany). The intra-assay and inter-assay coefficients of variation (CV) for the 25 (OH) vitamin D assay were less than 8% and 12%, respectively.

Statistical analysis

The median, first quartile, and third quartile were used to represent continuous variables that were not normally distributed. Differences between the two or three groups were analyzed using the Mann-Whitney U test and the Kruskal-Wallis test. An independent t-test was used to compare normally distributed data. Categorical variables were compared using the Chi-square test or Fisher's exact test. $P<0.05$ was considered significant. Spearman's rank correlation test was performed to determine the association between serum 25 (OH) vitamin D and the severity of MIS-C or COVID-19 pneumonia. Spearman correlation analysis was used to determine the correlation between laboratory results and serum 25 (OH) vitamin D levels. Statistical analyses were performed using the SPSS for Windows version 25 software (IBM, Armonk, NY, USA).

Results

This prospective observational study was performed on 51 patients with MIS-C, 57 patients with COVID-19, and 60 controls. When the sex and median age distribution of the groups were evaluated, there were no statistical differences between the three

groups (p=0.446 and p=0.089, respectively) (Table 1). The median serum 25 (OH) vitamin D level was 14.6 ng/mL in patients with MISC, 16 ng/mL in patients with COVID-19, and 21.1 ng/mL in the controls (p<0.001). In the subgroup comparison, serum 25 (OH) vitamin D levels were statistically significantly lower in patients with MIS-C compared with the controls (MIS-C *vs*. controls p<0.001; MIS-C *vs*. COVID-19 p=0.240; COVID-19 *vs.* controls p=0.058). Vitamin D insufficiency was present in 74.5% (n=38/51) of patients with MIS-C, 66.7% (n=38/57) of patients with COVID-19, and 41.7% (n=25/60) of the controls (Figure 1).

The characteristics of patients with MIS-C according to adequate/inadequate serum 25 (OH) vitamin D levels are shown in Table 2. Thirty-eight (74.5%) patients had vitamin D insufficiency and 13 (25.5%) had vitamin D sufficiency. The median age of patients with MIS-C was 8.8 [IQR (interquartile range) 5.6-12.3] years. Patients with adequate serum 25 (OH) vitamin D levels were younger compared with patients with inadequate serum 25 (OH) vitamin D (6 *vs*. 10.3 years; p=0.034) (Table 2). Thirty-three (64.7%) patients with MIS-C were male and 28.9% (n=15) were overweight-obese. The median length of hospital stay was 8 days in the inadequate vitamin D group and 5 days in the adequate vitamin D group (p=0.085). In the evaluation of admission symptoms (fever, fatigue, muscle ache, any gastrointestinal symptoms, conjunctival injection, mucous membrane changes, rash, arthralgia, any respiratory symptoms), there were no statistically significant differences between the adequate and inadequate vitamin D groups with MIS-C (p>0.05 for all).

The affected organ systems (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, or neurologic) were assessed in patients with MIS-C. The percentage of four or more affected organ systems was 39.2% among patients with MIS-C. It was found that the number of affected ≥4 organ systems was significantly higher in the group with inadequate vitamin D (47.4%, n=18) compared with the group with adequate vitamin D (15.4%, n=2) (p=0.041). When the correlation between the number of affected organ systems and serum 25 (OH) vitamin D levels was evaluated, there was a moderate negative correlation (r= -0.310; p=0.027). ICU stay was required in 15.7% (n=8) of patients with MISC, and all of these patients were in the inadequate vitamin D group (p=0.096). The Pediatric ICU (PICU) group had significantly lower serum 25 (OH) vitamin D levels compared with the non-PICU group (11.8 vs. 15.1; p=0.039) (Figure 2). Similarly, hypotension was noted in 34.2% (n=13) of patients, and shock developed in 26.3% (n=10) of patients; all of these patients were in the inadequate vitamin D group (p=0.023 and p=0.048, respectively). There were no deaths in the study population.

The characteristics of patients with COVID-19 are shown in Table 3. Thirty-eight (66.7%) of 57 hospitalized COVID-19 patients had vitamin D insufficiency. The median age was 11.8 years (IQR 3.8-15.7); 52.6% (n=30) of patients were male. When evaluating the clinical characteristics of the patients, dry cough was significantly more frequent in the group with inadequate serum vitamin D levels (73.7% *vs*. 47.4%, respectively; p=0.049). When evaluating the laboratory results, the lymphocyte count was significantly lower in the group with inadequate serum vitamin D levels (1300 *vs.* 2200 cells/uL, p=0.049). When evaluating the correlation between the severity of COVID-19 and serum 25 (OH) vitamin D, a weak negative correlation was found (r= -0.320, p=0.015) and the length of hospital stay (r= -0.304, p=0.022). The correlation between serum 25 (OH) vitamin D levels and laboratory results was evaluated. There was a moderate positive correlation between serum 25 (OH) vitamin D levels and aspartate aminotransferase levels (r=0.530; p<0.001), and a weak positive correlation with lactate dehydrogenase levels (r=0.269, p=0.043).

Discussion

To our knowledge, this is one of the first studies to analyze vitamin D levels in pediatric patients with MIS-C and a hospitalized COVID-19 group. In our study, the median serum 25 (OH) vitamin D level was inadequate in both patients with MIS-C and COVID-19 compared with the control group. It was lowest in the MIS-C group following COVID-19 and healthy controls (14.6 ng/mL in patients with MIS-C, 16 ng/mL in patients with COVID-19, 21.1 ng/mL in the control group).

There are insufficient studies on vitamin D status in patients with MISC in the literature. In a study by Darren et al., 16 of 18 (89%) patients with MIS-C had vitamin D insufficiency, and the mean 25 (OH) vitamin D level was 6.8 ng/mL. They also reported that the pediatric ICU group (n=12) had lower mean 25 (OH) vitamin D levels compared with the non-pediatric ICU group (8.9 *vs.* 5.6 ng/mL, respectively; p=0.110), but these results were not statistically significant (18). Zengin et al. compared the serum vitamin D levels of 34 MISC patients requiring intensive care with those of 34 control patients in a retrospective study. They discovered that patients with MISC had considerably lower serum 25 (OH) vitamin D levels than those without MISC (9 *vs.* 19 ng/mL) (19). Consistent with previous reports, 75% (n=38/51) of patients with MIS-C had either vitamin D deficiency or vitamin D insufficiency: the median 25 (OH) vitamin D level was 14.6 ng/mL. In our study, all patients who required ICU stay (n=8/51, 21%) were in the vitamin D insufficiency group. The pediatric ICU group had significantly lower 25 (OH) vitamin D levels than the non-pediatric ICU group (11.8 *vs*. 15.1 ng/mL, respectively). This finding warrants further investigation in larger MIS-C cohorts.

Although studies on vitamin D status in patients with MIS-C are limited, some studies focused on its relation to disease severity. In the study by Torpoco Rivera et al., the authors found that the seriousness of the MISC disease, especially cardiac involvement, was associated with severe vitamin D deficiency (25 (OH) vitamin D level <10 ng/mL) (20). In the study conducted by Mamishi et al., 122 patients with MISC were divided into two distinct groups (mild-moderate and severe). Mild-to-moderate MISC disease was identified in 97 of the patients, while severe MISC disease was identified in 25. Serum 25 (OH) vitamin D levels were considerably lower in patients with severe MISC (8.5 *vs.* 20.5 ng/mL) (21). In a review by Feketea et al., the authors concluded that serum vitamin D levels might help predict severe forms of MIS-C and that correction of abnormal levels in severe MIS-C could influence the development of the disease (22). Consistent with these speculations, we found a moderate negative correlation between serum 25 (OH) vitamin D and the number of affected organ systems in patients with MIS-C. These results primarily suggest that patients with inadequate vitamin D status had a more severe disease course. It is also speculated that vitamin D is an acute-phase response, and its blood level might decrease during the inflammatory process. MIS-C disease is known to occur as a

result of cytokine storms. It is thought that an excess of cytokines could lead to more severe inflammation and cause a further decrease in serum vitamin D levels. Like these speculations, in a study by Peterson et al., the authors found serum concentrations of tumor necrosis factor-alpha or C-reactive protein were inversely correlated with serum vitamin D concentrations (23). As another mechanism, it is worth noting that the need for active vitamin D, which has an anti-inflammatory effect, increases when a severe disease occurs, causing an enormous inflammatory process. Therefore, the turnover of vitamin D from serum in cells involved increasing immunomodulation, resulting in a decrease of inactive vitamin D from serum. From this point of view, the low serum vitamin D level in severe disease could be a consequence of severity and not a predisposing factor (24).

Apart from the well-known effect of vitamin D on calcium metabolism in humans, it regulates immune responses by increasing the production of anti-inflammatory cytokines, reducing plasma cells, decreasing the production of proinflammatory cytokines, and the release of immunoglobulins, thus stimulating the production of antimicrobial peptides in the respiratory system (7, 25). One such study by Katz et al. examined 887 adult patients with COVID-19, 87 of whom were vitamin D deficient. They found that patients with vitamin D deficiency were 4.6 times more likely to have positive COVID-19 status than patients without deficiency (95% confidence interval, 3.713-5.783) (26). Many studies conducted on adult patients showed a significant association between vitamin D deficiency and the severity of COVID-19 (26-28). In contrast, there are insufficient studies on children because of the milder clinical course of COVID-19. A study by Alpcan et al. retrospectively analyzed serum 25 (OH) vitamin D levels in 75 pediatric patients with COVID-19 and 80 healthy controls (29). The mean serum vitamin D level was significantly lower in the COVID-19 group than in the control group (21.5 *vs.* 28.0 ng/mL). They also showed that 84% of patients with COVID-19 had vitamin D insufficiency, as in the study by Molla et al., which was 82% (29, 30). Similar to previous reports, 66.7% of hospitalized patients with COVID-19 had vitamin D insufficiency in our population. Although the median serum vitamin D level was lower in the hospitalized COVID-19 group than in the control group, this was not statistically significant (16 *vs*. 21.1 ng/mL, respectively); we found a weak negative correlation between the severity of COVID-19 and serum vitamin D levels.

In a recent study comparing clinical features associated with COVID-19, according to vitamin D status, dyspnea, weakness, anosmia, headache, myalgia, and loss of taste were significantly more common in the insufficient vitamin D group (29). Regression analysis showed that low vitamin D level was a risk factor for the occurrence of dyspnea (Odds ratio= -0.268, 95% confidence interval: -15.920 to -1.406) (29). Our study showed that only dry cough was significantly more frequent in the group with insufficient vitamin D in patients with COVID-19 (73.7% *vs*. 47.4%). In a study evaluating laboratory results and serum vitamin D levels, vitamin D was positively correlated with leukocyte count, lymphocyte count, and platelet count. In contrast, it was negatively correlated with age and length of hospital stay (30). Our results showed that there was a moderate positive correlation between serum 25 (OH) vitamin D and aspartate aminotransferase and a weak positive correlation with lactate dehydrogenase levels. Importantly, we found a weak negative correlation between serum 25 (OH) vitamin D levels and length of hospital stay.

Limitations of the study

First, serum vitamin D levels were taken during the active inflammation phase. Serum vitamin D levels decrease during active inflammation in the human body. A more valid comparison would be possible if these patients' serum vitamin D levels before infection and inflammation were known. However, it is practically impossible to know in advance which patient will have MISC or COVID-19.

Conclusion

Our study sheds light on the relationship between vitamin D status in patients with MISC and COVID-19. It was observed that serum 25 (OH) vitamin D levels were correlated with the severity of MIS-C and COVID-19. However, it is unclear whether low vitamin D status is more common in patients with MIS-C than in the general population because there are no clinical trial data on this issue. Our study is the first to compare vitamin D levels in patients with MIS-C and healthy controls. Evaluation of serum vitamin D status of patients with MIS-C and COVID-19 before and during the disease will provide a better understanding of the pathophysiologic mechanism of this issue.

Acknowledgments

We want to express our gratitude to all the technicians working in our hospital who working in clinic of pediatric infectious disease.

Data Availability Statement: Additional data are available from the corresponding author on reasonable request.

Funding/Support: No funding was secured for this study.

Conflict of Interest Disclosures: All the authors have no example conflicts of interest to disclose.

Author contributions:

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

1. Guarantor of integrity of the entire study: Yildiz Ekemen Keles, Dilek Yilmaz Ciftdoğan, Eda Karadag Oncel
2. Study concepts and design: Yildiz Ekemen Keles, Dilek Yilmaz Ciftdoğan, Ahu Kara Aksay, Eda Karadag Oncel, Gulnihan Ustundag, Aslihan Sahin, Selin Tasar, Aysegul Elvan Tuz, Aslihan Arslan Maden, Ayfer Colak
3. Literature research: Yildiz Ekemen Keles, Dilek Yilmaz Ciftdoğan, Gulnihan Ustundag, Aslihan Sahin, Selin Tasar, Aysegul Elvan Tuz, Aslihan Arslan Maden, Eda Karadag Oncel
4. Critical review manuscript: Yildiz Ekemen Keles, Dilek Yilmaz Ciftdoğan, Eda Karadag Oncel
5. Experimental studies / data analysis: Yıldız Ekemen Keleş, Dilek Yilmaz Ciftdoğan, Eda Karadag Oncel, Gulnihan Ustundag, Aslihan Sahin, Selin Tasar, Aysegul Elvan Tuz, Aslihan Arslan Maden
6. Statistical analysis: Yildiz Ekemen Keles

7. Manuscript preparation studies: Yildiz Ekemen Keles, Dilek Yilmaz Ciftdoğan, Eda Karadag Oncel, Ayfer Colak, Ahu Kara Aksay
8. Manuscript editing: Dilek Yilmaz Ciftdoğan, Ayfer Colak, Eda Karadag Oncel, Yildiz Ekemen Keles

References

1. Karbuz A, Akkoc G, Bedir Demirdag T, Yilmaz Ciftdogan D, Ozer A, Cakir D, Hancerli Torun S, Kepenekli E, et al. Epidemiological, Clinical, and Laboratory Features of Children With COVID-19 in Turkey. Front Pediatr. 2021 May 7;9:631547. doi: 10.3389/fped.2021.631547.
2. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet. 2020 May 23;395(10237):1607-1608. doi: 10.1016/S0140-6736(20)31094-1.
3. Centers for Disease Control and Prevention (2020) Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19). https://www.cdc.gov/mmwr/volumes /69 /wr /mm6932e2.htm. Accessed August 2020
4. World Health Organization (2020) Multisystem inflammatory syndrome in children and adolescents with COVID-19. https://www.who.int/news-room/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. Accessed May 2020
5. Rowley AH. Understanding SARS-CoV-2-related multisystem inflammatory syndrome in children. Nat Rev Immunol. 2020 Aug;20(8):453-454. doi: 10.1038/s41577-020-0367-5.
6. Sacco K, Castagnoli R, Vakkilainen S, Liu C, Delmonte OM, Oguz C et al. Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19. Nat Med. 2022 May;28(5):1050-1062. doi: 10.1038/s41591-022-01724-3.
7. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflammation research : official journal of the European Histamine Research Society. Inflamm Res. 2014 Oct;63(10):803-19. doi: 10.1007/s00011-014-0755-z.
8. Hewison M. Antibacterial effects of vitamin D. Nat Rev Endocrinol. 2011 Jun;7(6):337-45. doi: 10.1038/nrendo.2010.226.
9. Boltz-Nitulescu G, Willheim M, Spittler A, Leutmezer F, Tempfer C, Winkler S. Modulation of IgA, IgE, and IgG Fc receptor expression on human mononuclear phagocytes by 1 alpha,25-dihydroxyvitamin D3 and cytokines. J Leukoc Biol. 1995 Aug;58(2):256-62. doi: 10.1002/jlb.58.2.256.
10. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006 Mar 24;311(5768):1770-3. doi: 10.1126/science.1123933.
11. Lemire JM, Adams JS, Kermani-Arab V, Bakke AC, Sakai R, Jordan SC. 1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity in vitro. J Immunol. 1985 May;134(5):3032-5.
12. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. Immunol. 2007 Aug 15;179(4):2060-3. doi: 10.4049/jimmunol.179.4.2060.
13. De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. 2000 Oct 2;192(7):1069-74. doi: 10.1084/jem.192.7.1069.
14. Dumache R, Enache A, Cut T, Paul C, Mihailescu A, Ionescu A et al. Deficiency of Vitamin D, a Major Risk Factor for SARS-CoV-2 Severity. Clin Lab. 2022 Mar 1;68(3). doi: 10.7754/Clin.Lab.2021.210327.
15. Aglipay M, Birken CS, Parkin PC, Loeb MB, Thorpe K, Chen Y, et al. Effect of High-Dose vs Standard-Dose Wintertime Vitamin D Supplementation on Viral Upper Respiratory Tract Infections in Young Healthy Children. JAMA. 2017 Jul 18;318(3):245-254. doi: 10.1001/jama.2017.8708.
16 World Health Organization (WHO). Clinical management of COVID-19. https://apps.who.int/iris/rest/bitstreams/1278777/retrieve (27 May 2020).
17. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab. 2016 Feb;101(2):394-415. doi: 10.1210/jc.2015-2175.
18. Darren A, Osman M, Masilamani K, Habib Ali S, Kanthimathinathan HK, Chikermane A, et al. Vitamin D status of children with Paediatric Inflammatory Multisystem Syndrome Temporally associated with Severe acute respiratory syndrome coronavirus 2 (PIMS-TS). Br J Nutr. 2021 May 12:1-26. doi: 10.1017/S0007114521001562.
19. Zengin N, Bal A, Goren TA, Bayturan SS, Alkan F, Akcali S. Serum Vitamin D Levels in Relation to Development of Multisystem Inflammatory Syndrome in Pediatric COVID-19. J Pediatr Infect Dis. 2022 Jun 17: 308-316. doi: https://doi.org/10.1055/s-0042-1756713.
20. Torpoco-Rivera D, Misra A, Sanil Y, Sabzghabaei N, Safa R, Garcia RU. Vitamin D and morbidity in children with multisystem inflammatory syndrome related to Covid-19. Prog Pediatr Cardiol. 2022 Mar 1:101507. doi: 10.1016/j.ppedcard.2022.101507.
21. Mamishi S, Olfat M, Pourakbari B, Eshaghi H, Abdolsalehi MR, Shahbabaie MA, et al. Multisystem inflammatory syndrome associated with SARS-CoV-2 infection in children: update and new insights from the second report of an Iranian referral hospital. Epidemiol Infect. 2022 Oct 18;150:e179. doi: 10.1017/S0950268822001522.

22. Feketea G, Vlacha V, Bocsan IC, Vassilopoulou E, Stanciu LA, Zdrenghea M. Vitamin D in Corona Virus Disease 2019 (COVID-19) Related Multisystem Inflammatory Syndrome in Children (MIS-C). Front Immunol. 2021 Mar 8;12:648546. doi: 10.3389/fimmu.2021.648546.
23. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond). 2008 Jul 24;5:10. doi: 10.1186/1476-9255-5-10.
24. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014 Jan;2(1):76-89. doi: 10.1016/S2213-8587(13)70165-7.
25. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol. 2012 Mar 1;188(5):2127-2135. doi: 10.4049/jimmunol.1102412.
26. Katz Joseph, Yue S, Xue W. Increased risk for COVID-19 in patients with vitamin D deficiency. Nutrition. 2021 Oct;90:111361. doi: 10.1016/j.nut.2021.111361.
27. De Smet D, De Smet K, Herroelen P, Gryspeerdt S, Martens GA. Serum 25(OH)D Level on Hospital Admission Associated With COVID-19 Stage and Mortality. Am J Clin Pathol. 2021 Feb 11;155(3):381-388. doi: 10.1093/ajcp/aqaa252.
28. Ye K, Tang F, Liao X, Shaw BA, Deng M, Huang G, et al. Does Serum Vitamin D Level Affect COVID-19 Infection and Its Severity?-A Case-Control Study. J Am Coll Nutr. 2021 Nov-Dec;40(8):724-731. doi: 10.1080/07315724.2020.1826005.
29. Alpcan A, Tursun S, Kandur Y. Vitamin D levels in children with COVID-19: a report from Turkey. Epidemiol Infect. 2021 Aug 10;149:e180. doi: 10.1017/S0950268821001825.
30. Karakaya Molla G, Ünal Uzun Ö, Koç N, Özen Yeşil B, Bayhan G. Evaluation of nutritional status in pediatric patients diagnosed with Covid-19 infection. Clin Nutr ESPEN. 2021 Aug;44:424-428. doi: 10.1016/j.clnesp.2021.04.022

Table 1. Characteristics and serum vitamin D levels between patients with MIS-C, hospitalized COVID-19 and the control group

	MIS-C	**COVID-19**	**Control group**	***p value***	***p value***		
					MIS-C vs COVID-19	**MISC vs Control**	**COVID-19 Control**
Patient number, n (%)	51	57	60	-	-	-	-
Age, years (IQR*)	8.8 (5.6-12.3)	11.8 (3.8-15.7)	10 (6.2-16.4)	0.089	-	-	-
Sex, n (%)							
Boy	33 (64.7)	30 (52.6)	35 (58.3)	0.446	-	-	-
Girl	18 (35.3)	27 (47.4)	25 (41.7)				
25 OH vit D levels (IQR)	14 (9.3-20)	16 (9.1-23.4)	21.1 (13.7-27.5)	<0.001*	0.240	<0.001	0.058
Vitamin D status, n (%)				0.001	0.373	<0.001	0.007
Vitamin D sufficiency	13 (25.5)	19 (33.3)	35 (58.3)				
Vitamin D insufficiency	38 (74.5)	38 (66.7)	25 (41.7)				

Table 2. Characteristics of the patients with MIS-C according to serum 25 hydroxy vitamin D levels

	All patients n=51	**Vitamin D insufficiency n=38**	**Vitamin D sufficiency n=13**	***p value***
Age, years, median (IQR)*	8.8 (5.6-12.3)	10.3 (6.1-13)	6 (2.6-10.3)	0.034
Overweight/obese n/total (%)	13/45 (28.9)	10/35 (28.6)	3/10 (30)	0.608
Sex, n (%)				
Girl	18 (35.3)	15 (39.5)	3 (21.1)	0.336
Boy	33 (64.7)	23 (60.5)	10 (76.9)	
25 (OH) Vit D levels, median (IQR)				
Girl	11.6 (6.7-18.2)	9.8 (6.3-12.7)	20.6 (20.5--)	-
Boy	14.4 (10.7-20.4)	13.3 (9.3-14.6)	23.1 (20.6-28.6)	-
Underlying medical condition, n (%)	17 (33.3)	14 (36.8)	3 (23.1)	0.502
Duration of hospitalization, median (IQR)	7 (4-11)	8 (5-13.2)	5 (3-8.5)	0.085
Number of organ systems involvements				
2-3	31 (60.8)	20 (52.6)	11 (84.6)	**0.041**
4≥	20 (39.2)	18 (47.4)	2 (15.4)	
Treatment				
Intravenous immunoglobulin n (%)	36 (70.6)	28 (73.7)	8 (61.5)	0.487*
Corticosteroids n (%)	31 (60.8)	26 (68.4)	5 (38.5)	0.098*
Anticoagulants n (%)	39 (76.5)	32 (84.2)	7 (53.8)	0.053*
Acetyl salicylic aside n (%)	5 (9.8)	3 (7.9)	2 (15.4)	0.591*
Inotropes n (%)	9 (17.6)	8 (21.1)	1 (7.7)	0.417*
Immunomodulatory therapy n (%)	4 (7.8)	4 (10.5)	0	0.561*
Need for oxygen n (%)	10 (19.6)	10 (26.3)	0	0.048*
Outcomes				
Hypotension n (%)	13 (25.5)	13 (34.2)	0	**0.023***
Extracorporeal membrane oxygenation n (%)	3 (5.9)	3 (7.9)	0	0.561*
Prone position n (%)	4 (7.8)	4 (10.5)	0	0.342*
Plasma exchange n (%)	4 (7.8)	4 (10.5)	0	0.295*
NIMV/MV n (%)	4 (7.8)	4 (10.5)	0	0.561*
Shock n (%)	10 (19.6)	10 (26.3)	0	**0.048***
Need for ICU n (%)	8 (15.7)	8 (21.1)	0	0.096*

* Interquartile range

Table 3. Characteristics of hospitalized patients with COVID-19 according to serum 25 hydroxy vitamin D levels

	All patients n=57	**Vitamin D insufficiency n=38**	**Vitamin D sufficiency n=19**	***p value***
Age, years, median (IOR)	11.8 (3.8-15.7)	13.3 (4.8-16.2)	5.5 (2.2-12.2)	**0.007**
Sex, n (%)				0.091
Girl	27 (47.4)	21 (55.3)	6 (31.6)	-
Boy	30 (52.6)	17 (44.7)	13 (68.4)	-
25 OH Vit D levels, median (IQR)				-
Girl	11.2 (8.1-19.5)	9.2 (7.6-13.7)	25.8 (23.5-32.9)	-
Boy	17.7 (12-25.3)	13.3 (9.8-16.7)	26.2 (22.4-31.6)	-
Underlying medical condition, n (%)	20 (35.1)	15 (39.5)	5 (26.3)	0.326
Duration of hospitalization, median (IQR)	5 (3-7)	5 (2.7-7)	4 (3-6)	0.274
Severity of COVID-19 pneumonia n (%)				0.292*
Mild	17 (29.8)	9 (23.7)	8 (42.1)	-
Moderate	22 (38.6)	15 (39.5)	7 (36.8)	-
Severe/Critical	18 (31.6)	14 (36.9)	4 (21.1)	-
Need for oxygen treatment n (%)	18 (31.6)	14 (36.8)	4 (21.1)	0.227
Need for ICU n (%)	5 (8.8)	3 (7.9)	2 (10.5)	1.000*

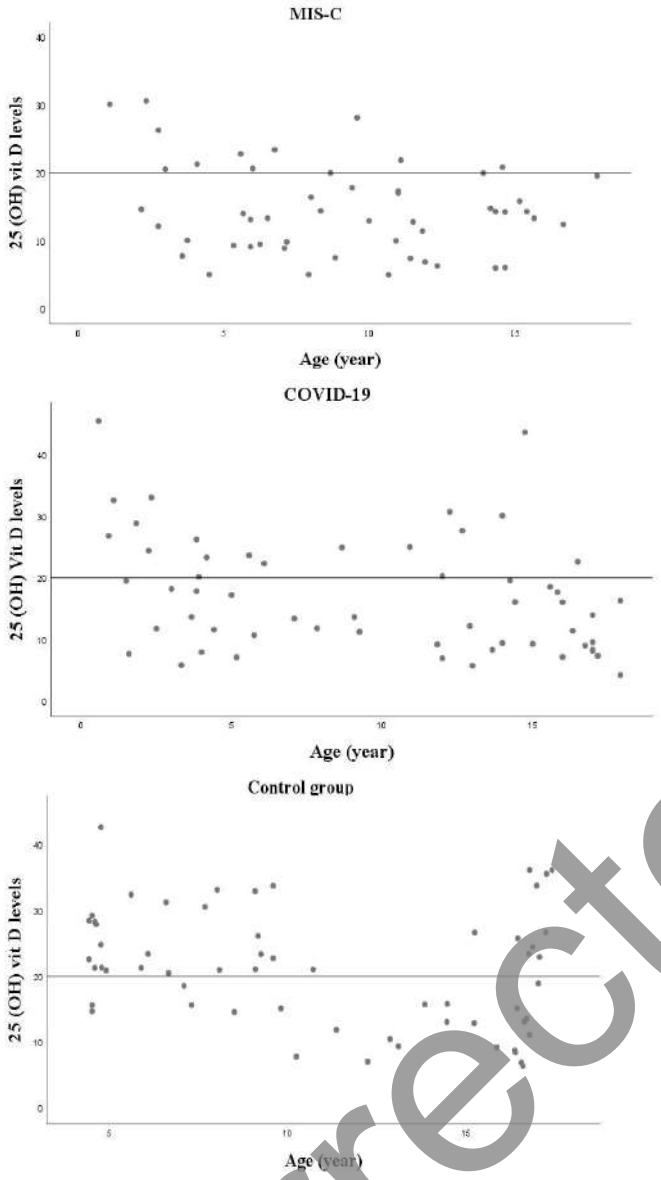

Figure 1. Serum 25 hydroxy vitamin D values in patients with MIS-C, COVID-19, and healthy controls