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The Clinical Significance of Vitamin D and Zinc Levels with Respect to Immune Response in COVID-19 Positive Children

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ABSTRACT

Aim: In this study, we aimed to evaluate serum vitamin D and zinc levels in children diagnosed with coronavirus disease 2019 (COVID-19).
Materials and methods: In this study, 88 children with COVID-19 disease and 88 healthy children aged 1–18 years were enrolled between 01 July 2021 and 30 October 2021 in the Pediatrics Clinic of Tekirdağ Çorlu State Hospital. Serum vitamin D and zinc levels have been measured and NCSS (Number Cruncher Statistical System) program has been utilized for statistical analysis.
Results: We included 88 COVID-19 positive pediatric patients [50% ($n = 44$) female] and 88 healthy children [48.86% ($n = 43$) female] in this study. The mean serum vitamin D levels of COVID-19 positive patients were statistically significantly lower than the control group ($p = 0.0001$). The zinc mean values of the study group were found to be statistically significantly lower than the control group ($p = 0.0001$). There was a statistically significant correlation between serum vitamin D and zinc values in all patient groups ($r = 0.245$, $p = 0.001$).
Conclusion: As a result, zinc and vitamin D levels were observed lower in COVID-19 patients than in healthy individuals. Since there is no defined treatment protocol for COVID-19 infection on children yet, zinc and vitamin D supplementation can be used as a supportive treatment in COVID-19 infection.

KEYWORDS: COVID-19, vitamin D, zinc, immune system, immunomodulatory

INTRODUCTION

In December 2019, the disease that first started with pneumonia epidemics of unknown origin in China and then spread rapidly all over the world was named coronavirus disease 2019 (COVID-19) [1, 2]. The first infection detected in the childhood age group was reported on 20 January 2020 [2]. In studies, the COVID-19 infection rate in children was lower than in adults [3]. While the complaints are nasal congestion, nasal discharge, headache, sore throat and diarrhea common in children with COVID-19, studies reported that some pediatric patients could develop

pneumonia, and more severe clinical symptoms may accompany [2].

There is no accepted treatment protocol yet to prevent this new virus or alleviate the disease severity. Therefore, vitamins, minerals and other trace elements play a critical function in keeping the immune system work healthy. Among these elements, vitamin D and zinc stand out. Micronutrient deficiencies can lead to immune dysfunctions by affecting the T-cell-mediated immune system and antibody responses [4].

Studies conducted in recent years have drawn attention to the relationship between vitamin D and systemic diseases [5, 6]. Vitamin D exerts an anti-inflammatory effect by affecting CD8+ T cells. It decreases proinflammatory cytokine (interferon gamma (IFN-γ) and Tumor necrosis factor-alpha (TNF-α)) secretion and, on the other hand, increases the anti-inflammatory cytokine (interleukin 5 (IL-5) and Transforming growth factor-beta (TGF-β)) secretion [7]. Vitamin D increases cellular immunity by reducing the cytokine storm caused by the innate immune system [8]. In addition, they have essential roles in B lymphocyte differentiation to plasma cells; and in the immune system through antibody production, antigen presentation and cytokine secretion [9]. Vitamin D inhibits antibody secretion and auto-antibody production in B lymphocyte cells and exerts an immune-modulatory effect [10]. Brown and Sarkar [11] study emphasized that vitamin D blocks the renin–angiotensin system by suppressing angiotensin-converting enzyme 2 (ACE2), and in this way, it can prevent lung damage due to COVID-19. Active vitamin D has an antiviral effect, especially against respiratory pathogens and is protective against influenza infections. It provides this effect by stimulating the secretion of cathelicidin, inducing over-secretion of proinflammatory cytokines, and suppressing natural killer cell (NK) function [12]. A study pointed out that COVID-19 cases increased in the winter months when the concentrations of 1,25 dihydroxy vitamin D3 [1,25(OH)2D3] were the lowest [13].

Zinc is the most abundant trace element in the body after iron and has a crucial role in many cellular functions, including the immune system [14], and its deficiency is associated with weakness in the immune system, growth retardation and cognitive impairment [15]. It has an important place, especially in antiviral immunity [14]. Zinc is critical for the normal development and function of nonspecific immunity mediating cells, such as neutrophils and NK [16]. Also, zinc induces the proliferation of CD8+ T cells, which modulate cytokine release and function as cytotoxic cells that can recognize and kill pathogens [17]. However, a study suggested that zinc may play roles in many pathways against COVID-19; regulation of inflammatory cytokines, induction of metallothionein, control of oxidative stress and phagocytosis [14]. A study stated that adequate zinc consumption minimizes the COVID-19 disease burden, affects the host's resistance to the infection and provides extra protection for COVID-19 infection [18].

In previous studies, it has been stated that vitamin D and zinc have a synergistic effect on the immune system. Deficiency of these nutrients in children will cause deterioration of mucosal epithelial cells, making them more susceptible to COVID-19. In our study, we aimed to compare the concentrations of these nutrients in healthy children and COVID-19 positive children.

MATERIALS AND METHODS

This study was conducted between 01 July 2021 and 30 October 2021, with the 'patient group' consisting of 88 patients (1–18 years of age), who applied to the Ministry of Health Tekirdağ Çorlu State Hospital Pediatrics Health and Diseases outpatient clinic. The study was accomplished following the ethical principles of the Declaration of Helsinki. This was a prospective cohort study conducted on the 'control group' of 88 healthy outpatients who applied to the Ministry of Health Tekirdağ Çorlu District State Hospital Pediatrics Outpatient Clinic with a gender distribution due to routine check-up controls. We calculated that we would need a sample size of 86 in each group to reliably (with a probability > 0.9) detect an effect size of $\delta \geq 0.5$, assuming a two-sided criterion for detection that allows for a maximum Type I error rate of $a = 0.05$. We picked two more patients in both groups, as some might have errors in their blood tests. We used a stratified random sampling method in the control group.

In both groups, unvaccinated subjects were included in the study. Patients who were diagnosed with any chronic disease, congenital disease, genetic

syndrome, immune deficiency, endocrinological, liver, kidney disease or inflammatory disease, patients with moderate and severe malnutrition, those using steroids and/or any food supplement containing vitamin D and zinc were not enrolled in the study. Individuals under the age of 1 and above the age of 18, and those without family consent were not included.

In this prospective study, we recorded laboratory results, imaging findings and clinical conditions on admission. Then we divided the patients into three groups by the results.

1. Asymptomatic group: patients who underwent polymerase chain reaction (PCR) testing only because of contact history and without any complaints.
2. Mild group: patients with nonspecific symptoms such as cough, fever, malaise and muscle pain.
3. Moderate-to-severe group: patients with pneumonia confirmed by physical examination and imaging (chest X-ray or computed tomography) with or without oxygen demand [19].

Patients of Turkish origin, who were in the asymptomatic group, were followed as out-patients, and living within the borders of Çorlu town were included in the study.

After obtaining approval from the families of the patients with real-time SARS-CoV-2 PCR test positive and healthy controls, blood samples were taken into pure tubes between 08.30 and 10.00 in the morning following 10–12 h of the fasting period. After the samples were coagulated at room temperature, we centrifuged for 10 min at 4000 rpm and isolated the serums. We stored the materials at −80°C in Tekirdağ Çorlu State Hospital Central Biochemistry Laboratory for analysis. All measurements were taken using a single blood sample during the same session.

Serum vitamin D levels were measured by electrochemiluminescence immunoassay method in the Cobas e 600 autoanalyzer device (Roche Diagnostics, F.Hoffmann-La Roche Ltd., Kaiseraugst, Switzerland). 25-hydroxyvitamin D [25(OH)D] levels were studied. Serum vitamin D reference ranges are 0–10 ng/ml severe deficiency; mild to moderate deficiency of 10–24 ng/ml; and 25–80 ng/ml was determined to be sufficient [20].

Serum zinc levels were measured in the Atomic Absorption Spectrophotometer (Perkin Emler AAS 800), flame photometric method and with the reference range of 60–150 µg/dl after the serum samples were diluted one-fourth with 5% glycerol.

Total serum calcium was determined by a colorimetric method and then corrected for albumin levels using the following formula:

$$\text{Corrected serum calcium} = \text{measured serum calcium} - 0.025 * (\text{serum albumin} - 40).$$

Statistical analysis

We used NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program for statistical calculations. Descriptive statistics are presented as mean and standard deviation. We used the Shapiro–Wilk test to assess data for normality. We used a Student's *t*-test to compare normally distributed variables between two groups, the Mann–Whitney *U* test to compare two groups with skewed data and the Chi-square test to compare two groups with categorical variables. Pearson correlation test was used to assess the relationship between variables. The results were evaluated at the significance level of $p < 0.05$.

RESULTS

We included 88 COVID-19 positive pediatric patients [50% ($n = 44$) female] and 88 healthy children [48.86% ($n = 43$) female] in the study. The mean age was 10.16 ± 4.64 years in the COVID-19 positive patient group and 8.9 ± 5.01 years in the control groups. There was no significant difference between the two groups by age and gender ($p = 0.085$ and $p = 8880$).

Among the COVID-19 positive patients, 39.77% had severe (below 10 ng/ml), 59.09% had a mild–moderate and 1.14% had slight vitamin D deficiency; however, these ratios were 19.32%, 56.82% and 23.86% in the control group, respectively. The mean serum vitamin D levels of COVID-19 positive patients were statistically significantly lower than the control group ($p = 0.0001$). Severe serum vitamin D deficiency in COVID-19 positive patients was statistically significantly higher than in the control group ($p = 0.0001$).

Table 1. Demographic and laboratory findings of the groups

	Control group *n*: 88 Mean ± SD/*n* (%)	Case group *n*: 88 Mean ± SD/*n* (%)	*p*-Value
Age	8.9 ± 5.01	10.16 ± 4.64	0.085[a]
Gender			
Male	45 (51.14%)	44 (50.00%)	0.880[b]
Female	43 (48.86%)	44 (50.00%)	
Calcium (8.6–10.3 mg/dl)	10.01 ± 0.31	9.83 ± 0.38	**0.001**[a]
Phosphorus (4–7 mg/dl)	4.34 ± 0.74	4.23 ± 0.68	0.284[a]
Magnesium (1.8–2.5 mg/dl)	2.12 ± 0.13	2.13 ± 0.26	0.689[a]
Alkaline phosphatase (30–120 U/l)	220.48 ± 100.61	183.44 ± 81.24	**0.008**[c]
Parathormone (10–65 pg/ml)	45.92 ± 39.32	46.74 ± 22.95	0.867
Vitamin D (ng/ml)	18.14 ± 8.81	11.73 ± 4.7	**0.0001**[a]
Vitamin D			
Severe deficiency (<10 ng/ml)	17 (19.32%)	35 (39.77%)	**0.0001**[b]
Mild to moderate (10–24 ng/ml)	50 (56.82%)	52 (59.09%)	
Sufficient (25–80 ng/ml)	21 (23.86%)	1 (1.14%)	
Zinc (μg/dl)	84.86 ± 19.65	70.32 ± 15.94	**0.0001**[a]
Zinc			
Normal (60–150 μg/dl)	82 (93.18%)	67 (76.14%)	**0.0001**[b]
Low (<60 μg/dl)	6 (6.82%)	21 (23.86%)	

[a]Independent *t* test.
[b]Chi-square test.
[c]Mann–Whitney *U* test.
The results were evaluated at the significance level of p <0.05. The results that are statistically significant are written in bold.

Table 2. Correlation tests of vitamin D and zinc variables of the groups

		Control group		Case group		All patients	
		Vitamin D	Zinc	Vitamin D	Zinc	Vitamin D	Zinc
Vitamin D	*r*		0.062		0.207		**0.245**
	p		0.565		0.053		**0.001**
Zinc	*r*	0.062		0.207		**0.245**	
	p	0.565		0.053		**0.001**	

Note: Pearson correlation test.
The results were evaluated at the significance level of p <0.05. The results that are statistically significant are written in bold.

Of the patients diagnosed with COVID-19, 23.86% and 6.82% of the control group had zinc deficiency. Also, the mean serum zinc levels of COVID-19 positive patients were statistically significantly lower than the control group ($p = 0.0001$).

There was no statistically significant difference in magnesium (Mg), phosphorus (P) and parathormone (PTH) levels between the two groups ($p = 0.284$; Table 1).

Calcium (Ca) levels of COVID-19 positive patients were statistically significantly lower than the control group ($p = 0.001$). In addition, the alkaline phosphatase (ALP) levels of COVID-19 positive patients were statistically significantly lower than the control group ($p = 0.008$; Table 1).

There was a statistically significant correlation between serum vitamin D and zinc values in all patient groups ($r = 0.245$, $p = 0.001$; Table 2).

DISCUSSION

Previous studies showed that serum of zinc and vitamin D levels in COVID-19 patients are lower than in healthy individuals [21]. Insufficiency of these nutrients will disrupt mucosal epithelial cells, making them more susceptible to COVID-19. In the study by Tezer and Bedir Demirdağ [22], ~1% of all COVID-19 cases in our country were in the childhood age group. Studies have shown that children with COVID-19 have a milder course than adults [23, 24]. However, concomitant diseases and dietary habits are of importance for the immune system [25]. In our study, we aimed to compare the concentrations of these elements in healthy children and COVID-19 positive children.

Camargo *et al.* [26] suggest that vitamin D is protective against respiratory tract infections, and supplementation of vitamin D reduces disease severity. The review of Alvares *et al.* [27] aimed to correlate 25(OH)D levels with the clinical prognosis of pediatric patients diagnosed with COVID-19. It was not possible to establish a relationship between serum levels of 25(OH)D and the clinical prognosis of COVID-19. Another study suggests that vitamin D levels in patients hospitalized in pediatric intensive care units due to sepsis were lower than in the control group [28]. Vitamin D reduces the production of proinflammatory cytokines and thus prevents the cytokine storm during COVID-19 infection [8], which has an important place in the clinical prognosis of COVID-19. A study reported that COVID-19 diagnosed children had lower vitamin D levels [29]. Another study suggested that the plasma concentration of 1,25(OH)2D3 in the patient group with severe COVID-19 infection was lower than in the group with the mild course, even though a study reported they did not observe fever in children with normal vitamin D levels [30]. Liu *et al.* [31] suggested that prostaglandin E (Pg E) drives the fever process, and vitamin D induces Pg E. In our study, 39.77% of COVID-19 positive patients were in the severe deficiency group with serum vitamin D below 10 ng/ml, and 59.09% were in the mild–moderate deficiency group. Similar to the studies in the literature, vitamin D levels were statistically lower than the control group [6, 8, 29].

Vitamin D is essential in serum calcium, phosphate and ALP levels. Also, studies indicated that vitamin D and calcium levels have a correlation [32, 33]. Holick [34] emphasized that vitamin D increases calcium absorption from the intestines. However, Sun *et al.* [35] study drew attention to hypocalcemia seen in COVID-19 positive patients. In our study group, serum calcium levels were lower in COVID-19 positive patients compared to healthy children, similar to this study.

Kiran *et al.* [33] and Shaheen *et al.* [36] have elaborated that phosphorus (P) and ALP were not correlated with serum levels of vitamin D. In this study, we also did not find any statistically significance between the phosphorus (P) and parathormone (PTH) and magnesium (Mg) levels of the control and study groups. In the human body, magnesium is primarily found in intracellular spaces (40%) or bones and teeth (60%). Approximately 0.3% of total body magnesium is placed in serum. Therefore, the total serum or ionized magnesium concentrations measured in plasma or serum are not reliable markers of total magnesium levels in the body [37].

Studies have suggested that magnesium may affect PTH synthesis and determines the number of vitamin D receptors. Therefore, a deficiency in magnesium levels can result in decreased PTH synthesis and secretion and a reduced number of vitamin D receptors expressed in target cells [38].

The COVID-19 virus enters the target cells by ACE2. Zinc suppresses ACE2, reducing viral interaction and protecting against COVID-19 infection [39]. In parallel with our study, a study reported that zinc levels in COVID-19 patients were statistically lower than in the healthy group [21, 40]. Heller *et al.* [40] reported that the mean zinc level was 71.7 ± 24.6 μg/dl in COVID-19 patients, while this level was higher (97.5 ± 29.4 μg/dl) in healthy individuals. In our study, while the mean zinc level was 70.32 ± 15.94 μg/dl in COVID-19 patients, it was higher (84.86 ± 19.65) in the healthy group. In addition, in a study conducted on patients infected with COVID-19, the zinc level in clinically more severe patients was lower than in mildly moderate patients [41]. In a comprehensive study conducted in Europe, Singh [42] stated a relationship between deaths due to COVID-19 and zinc levels. In another meta-analysis study, there was little evidence that supplementing with healthy foods would prevent respiratory infections, such as COVID-19. However, there is evidence that zinc supplementation may be beneficial in

children, and it seems that vitamin D supplementation provides some protection in adults [43].

There is an effect loop between zinc and vitamin D, as zinc can increase vitamin D activities, while vitamin D can affect zinc homeostasis. Zinc is needed for 1,25-dihydroxycholecalciferol to show biological activity. Zinc forms the 1,25-dihydroxycholecalciferol response element, which modulates the structure and binding domain of DNA; therefore, proper VDR structural conformation cannot be established without zinc. For this reason, the activity of vitamin D-dependent genes relies on zinc, making zinc an essential cofactor for vitamin D activity. Disruption of homeostasis of any of these nutrients can have undesirable effects leading to the possibility of numerous diseases, including but not limited to musculoskeletal disorders, cardiovascular disorders, immune dysfunction and healing defects [44]. Shams *et al.* [45] have found a positive correlation between serum zinc and serum vitamin D levels in children. In our study, we have observed that a statistically significant positive correlation existed between serum vitamin D values and zinc values in all patient groups.

Forty milligrams of zinc per day is considered a tolerable upper intake level and is unlikely to cause toxicity. Whether this same level of zinc intake can provide additional protection against COVID-19 infection, perhaps by increasing host resistance, is an area that needs further study [46].

Recommended upper limits for vitamin D intake are 100 µg/day (4000 IU/day) for adults and children 11–17 years old, 50 µg/day (2000 IU/day) for children 1–10 years old and 25 µg/day for infants (1000 IU) is considered appropriate. The higher threshold of vitamin D level of 50 nmol/l is supported in the literature by the evidence. This level can be safely achieved with supplementation of at least 800 IU per day (20 µg/day) [47].

Limitations

Our study is single-centered, and the number of patients is limited. Multicenter prospective studies can provide solid evidence to evaluate the relationship of zinc and vitamin D to disease incidence, contribution to treatment and clinical severity.

CONCLUSION

As a result, zinc and vitamin D levels were observed lower in COVID-19 patients than in healthy individuals. Since there is no defined treatment protocol for COVID-19 infection on children yet, zinc and vitamin D supplementation can be used as a supportive treatment in COVID-19 infection.

INSTITUTIONAL REVIEW BOARD APPROVAL

The study was granted approval by the Namık Kemal University Faculty of Medicine Clinical Trials Ethics Committee with an approval number of 2021.176.06.06 dated 29 June 2021 and informed consent has been obtained from all participants.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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