

## THE ROLE AND IMPORTANCE OF VITAMIN D IN PATIENTS WITH CORONA VIRUS DISEASE (COVID-19)

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### ABSTRACT

**Background:** Severe acute respiratory syndrome novel coronavirus (SARS-CoV-2) has rapidly progressed from an outbreak to a global pandemic, with new variants emerging rapidly. Coronavirus disease 2019 (COVID-19), a disease caused by SARS-CoV-2 infection, can lead to multiorgan damage. Due to the highly contagious and fatal nature of the virus, the priority of medical research has been to find effective treatments. Amid this search, the role of vitamin D in modulating various aspects of the innate and adaptive immune system has been debated.

The aim of the work: research on the role of vitamin D in the treatment and prevention of COVID-19.

**Patients and methods:** During this retrospective study, data were taken from the Registry of those tested for the presence of the SARS-CoV2 virus, Specialist Center “Health House” in Bijeljina. The research included 30 patients positive for the presence of the SARS-CoV2 virus and 30 patients negative for the presence of the SARS-CoV2 virus. The following parameters were observed in the research: age, gender and vitamin D values, data on the body temperature of the patients and the duration of the disease, as well as the obtained results of the inflammatory parameters (Leukocytes and C- reactive protein CRP). All patients were tested with the molecular method of polymerase chain reaction known as RT-PCR (Reverse transcription polymerase chain reaction). Separate samples were tested with Arhitectt Vitamin D reagent by the chemiluminescence method (CMIA), manufactured by Abbott Arhitectt and 1000sr.

**Results:** The concentration of vitamin D was lower in the COVID-19 group compared to individuals with the absence of the coronavirus disease. There is a tendency towards a negative correlation between the concentration of vitamin D and the duration of the disease in the group of patients COVID-19, as like as the concentration of vitamin D and body temperature. There is also a tendency towards a negative relationship between the concentration of vitamin D and parameters of inflammation (CRP, Le) in the COVID-19 group.

**Keywords:** vitamin D, COVID 19, inflammation, CRP

### INTRODUCTION

Severe acute respiratory syndrome novel coronavirus (SARS-CoV-2) has rapidly progressed from an outbreak to a global pandemic, with new variants emerging rapidly. Coronavirus disease 2019 (COVID-19), a disease caused by SARS-CoV-2 infection, can lead to multiorgan damage. Due to the highly contagious and fatal nature of the virus, the priority of medical research has been to find effective treatments. Amid this search, the role of vitamin D in modulating various aspects of the innate and adaptive immune system has been debated.

Local activation from 25(OH)D<sub>3</sub> to active 1,25(OH)<sub>2</sub>D<sub>3</sub> is of great importance in the context of the antiviral response in the respiratory system. Vitamin D receptors (VDRs) are constitutively expressed, and 1 $\alpha$  hydroxylase is present at high levels in macrophages and epithelia, among other cell types. 1,25(OH)<sub>2</sub>D<sub>3</sub> stimulation in lung epithelium has been shown to induce the expression of antimicrobial peptides such as cathelicidin 1 and defensin  $\beta$ <sub>4</sub>, in addition to the production of cathelicidin 1 in large amounts by macrophages and lower amounts in cell dendrites. Vit D- 1,25(OH)<sub>2</sub>D<sub>3</sub> and VDR signaling play similarly important roles

in the lung epithelium, and their roles in modulating responses to influenza and respiratory syncytial virus have been characterized.

It is possible that insufficient vitamin D levels contribute to cytokine storms, inadequate protection against epithelial cell apoptosis, and lack of epithelial cell repair, which can lead to adult respiratory distress syndrome. [1] It was also found that vitamin D and its biologically active hydroxyderivatives probably inhibit the activity of transmembrane serine protease 2 (TMPRSS2). This results in blocking the fusion between the viral spike protein and angiotensin converting enzyme 2 (ACE2), which is necessary for viral entry into the host cell. Biologically active molecules that prevent this interaction with serine protease have different synthetic pathways and perform different biological functions. Vitamin D moderates damage from proinflammatory mediators such as nuclear factor kappa-light-chain enhancer (NF- $\kappa$ B) and interleukin-17 (IL-17) in the skin after UVB exposure. If replicated in the systemic circulation, this mechanism is highly likely to moderate the effects of cytokine storms. However, as mentioned earlier, the route of delivery of vitamin D is a key factor that should be taken into account because it has a profound effect on circulating vitamin D derivatives.

In the context of SARS-CoV-2 infections, there have been few observational studies investigating the role of vitamin D. A large cross-sectional study examining the geographic distribution of COVID-19 in the USA examined the relationship between sunlight exposure and disease outcomes such as disease severity and death from COVID-19. [1] Similarly, a correlational analysis of data from 88 countries found that countries above the equator had fewer deaths from COVID-19 compared to distally distributed countries, suggesting a potential link between vitamin D (by latitude and sunlight exposure) and mortality from COVID-19. [2] In a cohort study of an elderly population patients with COVID-19, hospitalized in the United Kingdom, were observed to have lower serum vitamin D levels compared to healthy controls, and there was a notable correlation between deficiency vitamin D and the need for increased care. [3] This study also confirmed the findings that vitamin D deficiency is associated with an increased frequency of cytokine storms. [3] Similarly, in two studies of 107 and 4314 patients in Switzerland and Chicago, it was shown that patients positive for SARS-CoV-2 were more likely to have lower circulating concentrations of 25(OH)D. [4] A retrospective study on 185 patients with a diagnosis of COVID-19 showed that those with a low level of 25(OH)D in the serum are more likely to have poor outcomes, defined as the need for invasive mechanical ventilation or death. [5]

Ling et al, 2021 have shown that treatment with cholecalciferol or calcifediol improves outcomes such as ICU admission and mortality. [6] However, some studies have reported the opposite, negative results. Overall, the evidence supports the importance of the relationship between vitamin D and COVID-19, a relationship that requires continued investigation through large, nationally representative studies.

According to an insight into the available modern literature, we expected that the concentration of Vitamin D is lower in patients with the infection of COVID-19, and therefore that its value has a significant impact on the clinical presentation of patients and the outcome of the disease, as well as the length and severity of the duration of the disease, with the fact that all this needs to be further shed light on.

*The aim of the work:* research on the role of vitamin D in pathogenesis, treatment and prevention of COVID-19. The aim of the paper is to investigate the impact and importance of vitamin D in patients with the corona virus.

## PATIENTS AND METHODS

During this retrospective study, data were taken from the Registry of those tested for the presence of the SARS-CoV2 virus, Health Specialist Center "Health House" in Bijeljina, Bosnia and Herzegovina. The research included 30 patients positive for the presence of the SARS-CoV2 virus and 30 patients negative for the presence of the SARS-CoV2 virus. The following parameters were observed in the research: age, gender and vitamin D values, data on the body temperature of the patients and the duration of the disease, as well as the obtained results of the inflammatory parameters (Leukocytes and C- reactive protein - CRP). A sample of the patient's serum was used to determine the concentration of CRP. Determination of CRP values was performed on an automatic biochemical analyzer Roche Cobas C311, using the method of direct immunoturbidimetry. Reference value of CRP from 0.6 to 5 mg/L. The value of leukocytes was determined from a whole blood sample on the Mindray Bc 5800 automatic hematology analyzer, which works on the principle of volumetric impedance, flow cytometry and photometry. The number of leukocytes in the blood ranges from  $4 \times 10^9/L$ .

The data of all patients are anonymous. All patients were tested using the molecular method of polymerase chain reaction known as RT-PCR (Reverse transcription polymerase chain reaction). Separate samples were tested with Arhitectt Vitamin D reagent by the chemiluminescence method (CMIA), manufactured by Abbott Arhitect and 1000sr. Reference values of Vitamin D are from 75 to 150 nmol/L.

All research results are processed as statistical research results, differences between the obtained results were treated statistically, by accepting or rejecting the above working hypotheses. To test a statistically significant difference between respondents, we use the Student's test and the correlation test. Statistical significance was taken at the  $P < 0.05$  level.

## RESULTS

The concentration of vitamin D was lower in the group of patients with the coronavirus disease (COVID-19) compared to individuals with the absence of the coronavirus disease. COVID-positive cases had a statisti-

cally significant ( $t=7.03$ ,  $p<0.000$ ) lower concentration of vitamin D (mean= 40.1 nmol/L) compared to people without corona virus disease where the average concentration was significantly higher and amounted to 78.0 nmol/L.

We also assumed that there is a negative correlation between the concentration of vitamin D and the duration of the disease in the group of patients with the coronavirus disease (COVID-19), however Pearson's linear correlation did not prove a statistically significant negative correlation ( $r= -0.120$ ) between the analyzed variables with a probability of  $p < 0.528$ . There is still a tendency towards a negative correlation between the concentration of vitamin D and the duration of the disease in the group of patients with the coronavirus disease (COVID-19), as like as the concentration of vitamin D and body temperature in the group of patients

with the coronavirus disease (COVID-19). There is also a tendency towards a negative relationship between the concentration of vitamin D and parameters of inflammation (CRP, Le) in the group of patients with coronavirus disease (COVID-19) in this research, a statistically significant negative linear correlation between the height of the body temperature and the height of vitamin D in patients with coronavirus disease was not proven, as shown in tables.

Our fourth working hypothesis claimed that there is a negative correlation between vitamin D concentration and inflammation parameters (CRP, Le) in the group of patients with coronavirus. Regardless of the association of the variables, statistical significance was not reached.

All above mentioned is shown tables and image.

**Table 1.** Vitamine D concentrations in Corona virus positive and negative patients

	Negative			Positive			p
	N	95% CI Mean	Standard Deviation (SD)	N	95% CI Mean	Standard Deviation (SD)	
Vitamine D mol/L	30	78,0±9,37	26,2	30	40,1±4,9	13,7	<b>0,000</b>

**Table 2.** Leukocytes, CRP and length of disease in COVID-19 and COVID negative patients with vitamin D level (<75 nmol/L)

Cases						
COVID negative			COVID positive			P
Mean	SD	N	Mean	SD		
6,98	1,92	30	6,36	1,87	0,297	
1,70	2,29	30	4,57	5,62	0,013	
/	/	30	15,43	10,39	/	

**Table 3.** Frequency of high temperature in patients with COVID -19 depending of vitamin D levels

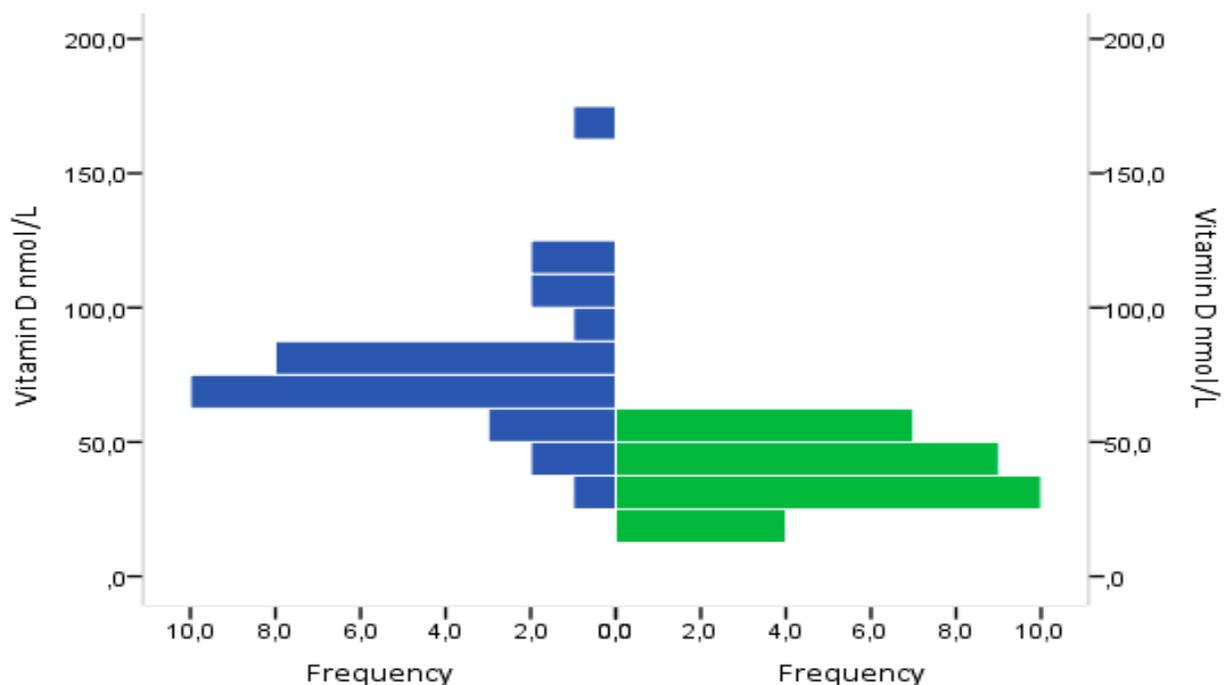
Vitamin D concentrations	Body temperature				p
	Normal		High		
	N	%	N	%	
Very low (< 30 nmol/L)	2	25,0%	6	75,0%	0,157
Low (< 50 nmol/L)	9	60,0%	6	40,0%	0,439
Insufficiency -failure (<75 nmol/L)	3	42,9%	4	57,1%	0,705
Appropriate - normal (75 - 125 nmol/L)	0	0,0%	0	0,0%	/

**Table 4.** Correlation of vitamin D, leukocytes, CRP and length of disease in COVID positive group

		Leucocytes	CRP	Length of disease (days)
Vit D nmol/L	r	0,301	0,293	-0,120
	p	0,106	0,116	0,528
	N	30	30	30

**Table 5.** Tests of Pearson linear correlations between COVID positive and COVID negative groups

Variables	N	COVID negative (r)	COVID positive (r)	p
Leukocytes vs. Vitamin D	30	0,023	0,301	-0,145
CRP vs. Vitamin D	30	0,119	0,293	0,252



**Figure 1.** vitamin D distribution in COVID positive (dark blue color) and COVID negative (green color) cases

**DISCUSSION**

COVID 19 is a disease that appeared in 2019, and as such is still insufficiently researched. However, the treatment of this disease is the subject of study by scientists around the world, as we had the opportunity to see from the aforementioned studies that we highlighted. In our study, although the number of subjects was small, more precisely 60 subjects, the results obtained showed that the concentration of Vitamin D was lowered in patients with coronavirus disease, which corresponds to the results of numerous studies mentioned above.

Numerous studies have written about the immunomodulatory effect of Vitamin D in both the innate and

the acquired immune response, but considering that our research is connected to COVID-19, in this case we will refer to study conducted in 2020, in which she described that Vitamin D can reduce the risk of respiratory infections (including the SARS COV-2 virus). [7,8] The cells of the respiratory tract have receptors for Vitamin D, and in this way they can activate it to act autocrine and paracrine, which mainly preserves the integrity of the respiratory epithelium and reduces the excessive production of cytokines, enabling a balanced immune response to infection and preventing a cytokine storm (which results in acute damage lungs that occurs in the most severe cases of coronavirus disease). We emphasize again that, in our study, the positive cases had a statistically significant ( $t=7.03, p<0.000$ )

lower concentration of Vitamin D where the average value was 40.1 nmol/L, compared to patients who were negative, where the concentration of vitamin D was significantly higher (to 78.8 nmol/l). Based on the obtained statistical results, we can confirm the hypothesis that the concentration of vitamin D was lower in CoviD-19 group, compared to individuals with the absence of coronavirus disease ( $p < 0.001$ ). We could attribute this to the positive and beneficial immunomodulatory effects of vitamin D on immunity and the body's immune response. Vitamin D itself, with its pleiotropic, immunological and hormonal effects, which are described in detail in the introductory part, can have a beneficial effect on the prevention of COVID-19 on the one hand, but also on a more favorable clinical course and a better prognosis in case of an already existing infection on the other hand.

The duration of the disease in patients was monitored in subjects with a reduced concentration of vitamin D, and the severity of the disease was measured by the level of leukocytes in the blood, CRP and elevated body temperature.

Based on the results of the t-test, we came to the conclusion that COVID-positive patients with low vitamin D concentrations had statistically significantly ( $p < 0.013$ ) higher CRP values, compared to patients who are negative, but when it comes to leukocyte values, the t-test did not show statistical significance. It is possible that in this case, a more detailed analysis of the parameters of the differential blood count and individual lineages would give more precise results with statistical significance and eventual correlation. COVID-positive patients who had a reduced concentration of vitamin D had an elevated body temperature (as many as 75%). In our study, given the relatively small sample, it is more difficult to establish significance, but it can be observed that the associations between the concentration of vitamin D, CRP, leukocytes and elevated body temperature are more pronounced in patients with coronavirus disease compared to patients without absence from coronavirus disease (there is a tendency towards correlation).

Vitamin D and COVID -19 are a very current and interesting topic, a recently published study by a team of scientists from the Azrieli Faculty of Medicine of Israel's Barr-Ilan University and Israel's Galilee Medical Center suggests a link between Vitamin D deficiency and the severity of the disease caused by the infection of COVID 19. [9] Aforementioned study investigated the concentration of Vitamin D in 1176 patients who were hospitalized with a positive PCR test. According to them, patients with Vitamin D deficiency were 14 times more likely to have a more severe clinical form of the coronavirus disease. The conclusion of the Israeli scientists in this study was that vitamin D deficiency is one of the main risk factors for a severe clinical presentation, disease progression and even death.

The significance of the research lies in the fact that vitamin D could be used for the prevention and treatment of the disease itself. It is a widely available medicine, which is not expensive, and is therefore easily accessible to the wider population. In one study, out of

50 patients suffering from coronavirus disease who received Vitamin D, only one patient ended up in intensive care, and in another group of patients who did not receive Vitamin D, as many as 50% of patients required intensive therapy and care. [10]

It is possible that insufficient vitamin D levels contribute to cytokine storms, inadequate protection against epithelial cell apoptosis, and lack of epithelial cell repair, which may lead to adult respiratory distress syndrome. It was also found that vitamin D and its biologically active hydroxyderivatives probably inhibit the action of TMPRSS2. This results in blocking the fusion between the viral spike protein and ACE2, which is necessary for viral entry into the host cell. Biologically active molecules that prevent this interaction with serine protease have different synthetic pathways and carry out different biological functions, and would  $1\alpha,25(\text{OH})_2 \text{D}_3$ ,  $25(\text{OH})\text{D}_3$ ,  $1\alpha,20\text{S}(\text{OH})_2 \text{D}_3$ ,  $20\text{S},23\text{R}(\text{OH})_2 \text{D}_3$ ,  $20\text{S},23\text{S}(\text{OH})_2 \text{D}_3$  and  $1\alpha,20\text{S},23\text{S}(\text{OH})_3 \text{D}_3$  could be promising TMPRSS2 inhibitors. There is evidence that vitamin D attenuates damage to proinflammatory mediators such as NF- $\kappa$ B and IL-17 in the skin following UVB exposure. If replicated in the systemic circulation, this mechanism is highly likely to moderate the effects of cytokine storms. However, as mentioned earlier, the route of vitamin D delivery is a key factor to consider as it has a profound effect on circulating vitamin D derivatives. [11] In addition, the study by Glinsky et al. 2020, investigating the targets of SARS-CoV-2 using genetic studies confirmed the significant role of vitamin D in COVID-19. [12] They investigated vitamin D as a putative repressor of ACE2 expression and found that vitamin D inhibited ACE2 expression in human bronchial smooth muscle cells by VDR and other transcription factors. Of the 332 genes encoding SARS-CoV-2 prey proteins, vitamin D affects the expression of 84 (25%).

We already mentioned investigation of Baktash et al. (2021) who concluded that vitamin D deficiency is associated with an increased frequency of cytokine storms as well as in other similar investigations. [1,3] Aforementioned retrospective study of 185 patients diagnosed with COVID-19 showed that those with low serum  $25(\text{OH})\text{D}$  levels were more likely to have poor outcomes, defined as the need for invasive mechanical ventilation or death. [5]

Progress in this field of research is, to a large extent, limited by the rapidly evolving nature of the virus, as well as its effects on the immune system, which are still not fully understood. The phenomenon of more infectious or virulent strains of the virus causing adverse outcomes even in younger populations thought to be more immunologically resistant calls for reexamination of what little is known. Current evidence, although largely supportive of the hypothesis supporting the beneficial importance and effect of vitamin D, is not absolute and is still somewhat controversial.

In addition, the underlying cause of vitamin D deficiency and, therefore, the immune dysfunction seen in COVID-19 is still debated. A plausible explanation has also been proposed that diabetes and obesity (both accompanied by vitamin D deficiency states) result in in-

creased mortality from COVID-19, rather than vitamin D deficiency itself. Others eg. Stroehlein et al. 2021 are speculating about “healthy user effect”- healthy people who eat properly (in terms of adequate micro- and macronutrient intake) and exercise spend more time outdoors and therefore have higher levels of vitamin D.[14] This supports correlations between vitamin D deficiency and poor outcomes after COVID-19, but not and causal relationships.

Across the clinical trials reported here, it is apparent that there is considerable clinical and methodological heterogeneity, mainly due to different supplementation regimens and outcomes, as well as varying vitamin D status among participants. Therefore, there is a need for additional well-designed, adequate research and studies to determine the role of vitamin D in COVID-19. As noted in a recent Cochrane review, there are currently at least 21 ongoing studies that may shed light on this topic in the near future. [14] In addition, it is important to acknowledge data limitations in observational studies, including our study, which has a relatively small sample. There are numerous reasons why the conclusions of many previous studies are not representative.

As we have pointed out, COVID -19 is a disease that appeared in 2019, and as such is still insufficiently researched. However, the treatment of this disease is the subject of study by scientists around the world, as we had the opportunity to see from the aforementioned studies that we highlighted. In our study, although the number of subjects was small, more precisely 60 subjects, the results obtained showed that the concentration of Vitamin D was lowered in patients with coronavirus disease, which corresponds to the results of numerous studies mentioned above.

Special emphasis should be placed on the insufficient research of treatment with vitamin D, considering that it is a supplement that is affordable, easily available, and at the same time has numerous advantages for patients as well as for society from a socioeconomic point of view. This is where the greatest clinical significance of our research, as well as current similar research, lies. The research is primarily important because it tries to shed light on the role and importance of vitamin D, but more research will be needed to look at this problem from all angles and solve it as precisely and effectively as possible. The limitation of our study is small sample of patients.

Conflicts of interests: none

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