

Rapid elevation of vitamin D level reduces C-reactive protein level in COVID-19 patients with vitamin D insufficiency

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Abstract

Background: According to newly published studies, patients with Coronavirus Disease 2019 (COVID-19) have significantly lower serum 25-hydroxyvitamin D 25(OH)D levels than those without the SARS-CoV-2 infection. Vitamin D insufficiency may be a predictor of poor prognosis in COVID-19 patients. Vitamin D supplementation is related to improved clinical outcomes in terms of intensive care unit (ICU) admission and death, particularly in individuals with moderate-to-severe forms of COVID-19 requiring hospitalization.

Materials and methods: This placebo controlled five-day study was performed on 100 hospitalized COVID-19 patients with vitamin D insufficiency randomized into two groups. Vitamin D in the form of a sublingual sprayable microemulsion (LYL love your life® sunD3 LYLmicro™) was given three times daily after breakfast, lunch, and dinner (daily dose 12,000 IU) to 52 patients with blood vitamin D levels below 30 ng/ml. 48 patients in the control group received a placebo spray in the same daily regiment.

Results: We observed a statistically significant increase by 8.6 ng/ml in median vitamin D level after five days of high-dose vitamin D3 spray therapy. 90.2% of subjects reached normal serum levels of vitamin D. There was a strong correlation between changes in vitamin D and C-reactive protein (CRP) levels in individuals with moderately severe disease ($p < 0.05$), while mild and severe cases showed no statistical significance comparing case and control groups. There were no statistically significant changes in ferritin and interleukin-6 (IL-6) levels.

Conclusions: In this study we demonstrated the possibility to rapidly increase circulating levels of vitamin D just in 5 days of high-dose intervention and found a statistically significant decrease of CRP levels in patients with moderately severe COVID-19. We were not able to detect statistically significant changes in ferritin and IL-6 levels during 5-day vitamin D intervention.

Keywords: COVID-19, vitamin D insufficiency, vitamin D spray, CRP.

BACKGROUND

Vitamin D and prognosis. Several studies suggest that vitamin D insufficiency may be a predictor of poor prognosis in COVID-19 patients [1-9]. Vitamin D supplementation, as demonstrated by R. Pal et al, is related to improved clinical outcomes in terms of ICU admission and death, particularly in individuals with moderate-to-severe COVID-19 necessitating hospitalization [1, 10]. In the study the term *vitamin D insufficiency* is used to describe the clinical manifestation of COVID-19 in presence of low blood vitamin D level. The observational study done by Kaufman et al showed that the rate of SARS-CoV-2 positivity is both directly and inversely linked with the amount of circulating 25(OH)D, a connection that holds across latitude, race/ethnicity, sex, and age [8]. Severity of clinical disease and mortality in COVID-19 upturns in case of vitamin D insufficiency. Vitamin D insufficiency patients have a more pronounced inflammatory response, which is accountable for the increase in morbidity and mortality [9].

Low vitamin D levels and prognosis. The clinical studies in individuals with a 25(OH)D level of less than 20 ng/ml demonstrated an increased risk of COVID-19 compared to individuals with a vitamin D level of 30–34 ng/ml [3-8]. An extensive retrospective cohort study published by Oristrell et al showed that those who received cholecalciferol or calcifediol supplementation and achieved serum 25OHD levels of 30 ng/ml had superior COVID-19 treatment results [11].

Vitamin D and contradictory findings. Numerous research is underway to determine the benefits of vitamin D on individuals with COVID-19 [13]. However, researchers produced contradictory findings. After high-dose intramuscular D3 treatment in the early period of COVID-19, no difference in duration of hospital stay, endotracheal intubation, or death rates in critical patients in the ICU has been seen in the study of Güven and Gültekin [14]. Another study had similar findings, showing no link between vitamin D levels and COVID-19 severity or death in 341,484 UK Biobank individuals [15]. The Mendelian randomization study by Butler-Laporte et al found no evidence to indicate a relationship between 25OHD levels and COVID-19 susceptibility, severity, or hospitalization in a two-sample MR investigation [16]. In the systematic review and meta-analysis done by Basstne and colleagues, where they analyzed 31 peer-reviewed observational studies, found that while the existing data to date, derived primarily from low-quality observational studies, may be interpreted as indicating a tendency toward a link between low blood 25(OH)D levels and COVID-19-related health outcomes, this link was not found to be statistically significant. A possible preventive effect of calcifediol supplementation on COVID-19-related ICU admissions could be found [17].

Vitamin D and CRP. At the same time, it is worth mentioning contradictory findings as well in response to inflammatory markers. Higher levels of CRP during COVID-19 have been found to have greater complications and a more severe case of COVID-19 overall [18]. Several studies, which are in line with our study, have shown an inverse association between vitamin D and CRP [19-20]. One study showed inverse correlation between vitamin D and CRP, but more importantly highlighted that said inverse correlation is stronger in patients who have inflammatory diseases compared to patients who do not have those [20]. Yet, another study shows that there is not a noticeable inverse relation between vitamin D supplementation and inflammatory biomarkers

specifically in asymptomatic adults [21]. The study where vitamin D and inflammatory marker CRP were compared in people aged 60 years and older, showed that CRP levels were lower in patients with overall higher vitamin D levels. These findings with vitamin D intervention study could help answer the question if vitamin D decreases CRP levels or if CRP decreases vitamin D levels [22].

A single-center study studying COVID-19 and inflammatory markers showed that in the first wave of COVID-19 inflammatory markers were higher than in the second wave most likely due to changed guidelines on treating patients [23].

Vitamin D and ferritin and IL-6. Vitamin D may affect ferritin and IL-6 levels as it influences iron metabolism and erythropoiesis. Iron is one of the essentials for vitamin D synthesis [24]. There is a probability that vitamin D down regulates mRNA expression of hepcidin levels. It's believed that antimicrobial hepcidin peptides influence iron absorption and release through activating and suppressing ferroprotein which is a cellular iron exporter [25]. In a systematic review done by Arabi et al that included a total of 14 reports and 1385 individuals with mean baseline of 25(OH)D levels ranging between 10 and 30 ng/ml observed that vitamin D supplementation in 11 of the included studies did not improve ferritin concentrations overall. The study concluded that vitamin D supplements had no significant effect on ferritin levels while having positive effects on transferrin saturation and iron status [26]. However, a prospective observational study by Singh et al that included 343 COVID-19 positive asymptomatic and 59 ICU patients, argued that inflammatory markers such as serum ferritin and IL-6 were significantly elevated in critically ill patients and high levels of vitamin D correlate with reduced levels of IL-6 [27]. In another cross-sectional study by Teama et al that included 124 patients diagnosed with COVID-19 a negative correlation was observed between vitamin D and ferritin. Study concluded that vitamin D deficiency and low serum 25(OH)D levels significantly and negatively correlated with ferritin. This indicates that vitamin D might have a beneficial role on the systemic inflammatory state of COVID-19 [28].

METHODS

The aim of this study was to investigate the impact of the high dose vitamin D supplementation on laboratory markers of systemic inflammation such as CRP, ferritin and IL-6 in hospitalized patients with COVID-19. It was performed at the Pauls Stradiņš Clinical University Hospital (PSCUH) from February till July 2021. The blood samples were analyzed at the Joint Laboratory of the hospital. The study protocol was approved by the local ethics committee. A written consent was obtained a day before intervention began. Patients with confirmed SARS-CoV2 infection (PCR) were randomly divided into two groups: the intervention group received vitamin D in the form of a sublingual sprayable microemulsion (LYL love your life® sunD3 LYLmicro™) of 4,000 IU three times daily after breakfast, lunch and dinner; the control group received the same regiment of placebo spray.

A total of 100 inpatients were selected for the study, 48 patients in the vitamin D group and 52 in the control group. All patients received standard care for COVID-19 and existing comorbidities (diabetes, arterial hypertension, etc) according to hospital approved protocol. The primary outcome was defined as the change in the level of inflammatory markers and the severity of the disease.

Only those patients whose vitamin D level was below 30 ng/ml were included in the study. Patients with mental health problems, eGFR \leq 30 ml/min, vitamin D level \geq 30

ng/ml, pregnant women and any other illness or condition that the researcher deemed may interfere with the results were excluded. All individual patient data obtained during the study was coded and was available only to the study participants. The duration of the pharmaceutical intervention was 5 days.

Population. Disease severity was defined by the blood oxygen saturation level. Mild clinical manifestation was characterized by $\text{SpO}_2 \geq 94\%$, moderate $90\% \leq \text{SpO}_2 < 94\%$ and severe - $\text{SpO}_2 < 90\%$. Age, BMI, GFR, vitamin D and COVID-19 severity were considered as randomization parameters. 25(OH)D, ferritin and IL-6 were analyzed by chemiluminescence immunoassay (Atellica IM 1300 Analyzer, Siemens) while C-reactive protein (N: 0–5 mg/l) was processed by a turbidimetric method using Atellica CH 930 Analyzer, Siemens.

Statistical analysis. Shapiro – Wilk ($n < 50$ in the group) or Kolmogorov-Smirnov ($n > 50$ in the group) tests were used to evaluate whether the data follows the normal distribution with statistically significant PN.

The difference between groups with a nominal distribution was determined by the chi-squared (χ^2) criterion method. A statistically significant difference between groups was considered at $P_\chi < 0.05$. Cramer's V was chosen to determine the relationship between nominal data: above 0.10 - weak relationship, above 0.30 - medium, and above 0.50 - strong relationship.

T-test (statistical confidence P_T) was used to determine differences between group numerical values (mean or median) if data from both groups were normally distributed. The Mann-Whitney test (P_{Mv}) was applied if at least one group was not normally distributed. ANOVA test (P_F) or Kruskal-Valis (P_{kv}) test was used if more than two groups were compared.

The relationship between nominal and numerical indicators was determined using the eta (η) indicator: up to 0.2, the relationship was considered very weak; from 0.2 to 0.4 weak; from 0.4 to 0.7 moderately tight; from 0.7 to 0.9 tight; above 0.9 very tight, and 1.00 perfect.

In all tests, the statistically significant difference between groups was considered when $P < 0.05$. All tests were performed using IBM SPSS Statistics version 25.0.

RESULTS

The mean age in the placebo group was 67.1 ± 13.5 years (Table 1), with an interval of 34 to 88 years. The confidence interval (CI 95%) for the mean was 59.2 to 67.0 years. The mean age in the vitamin D group was 63.1 ± 13.9 years (Table 1), with an interval of 34 to 90 years. Both groups were in normal distribution according to the specific indicator (Table S1, supplementary).

In the case of the B group only responders (true case group), and in the A group only nonresponders (true placebo group) were selected. Inclusion/exclusion was determined by statistically significant changes in vitamin D levels according to the coefficient of variation (CV) of the laboratory equipment (CL5I DocumentEPQ5-A3). Those participants whose vitamin D level on the sixth day was higher than on the first day were considered to be responders. The equation used for inclusion was:

$$\begin{aligned} \text{CvitD2} &> \text{CvitD1} + \text{CV} - \text{responders} \\ \text{CvitD2} &< \text{CvitD1} + \text{CV} - \text{nonresponders} \end{aligned}$$

Where

CvitD2 - level of vitamin D on the 6th day

CvitD1 - level of vitamin D on the 1st day

CV - coefficient of variation

90.2% of all participants in group B fit the criteria of the true case group (Table 1). Whereas only 34 participants (70.7%) in the placebo group showed no increase in vitamin D levels in the blood.

Table 1. True case and true placebo groups

Group	Total amount of patients	Response to vitamin D therapy			
		CvitD2 > CvitD1+CV		CvitD2 < CvitD1+CV	
		Amount	%	Amount	%
A (placebo)	48	14	29.2	34	70.7
B (vitamin D)	51	46	90.2	5	9.8

Severity of COVID-19 cases

The distribution of patients in both groups by severity of disease was statistically similar (Table 2). The difference between groups was not statistically significant ($p=0.06$).

Table 2. Distribution of patients according to severity of disease COVID-19

Disease severity	Group				Statistical analysis	
	A (placebo)		B (Vitamin D)		P_{χ}	V
	Amount	%	Amount	%		
Mild	8	23.53	8	17.39	0.06	0.27
Medium	21	61.76	20	43.48		
Severe	5	14.71	18	39.13		
Total	34	100%	46	100%		

P_{χ} – statistical reliability of chi-squared (χ^2) criterion method; V - Cramer's V index for relationship

Changes in vitamin D levels

The level of vitamin D increased on average by 8.69 ± 7.63 ng/ml in group B, also the median in group B increased by 5.82 ng/ml with an IQR (interquartile range) of 8.37 ng/ml indicates an increase in value. The change in vitamin D levels in group B over 5 days was considered statistically significant $PV = 2.72 \times 10^{-14}$ (Table 3 and Figure 1). However, it should be noted that the delta values of vitamin D level in 5 patients (9.8%) from group B didn't rise above the coefficient of validation.

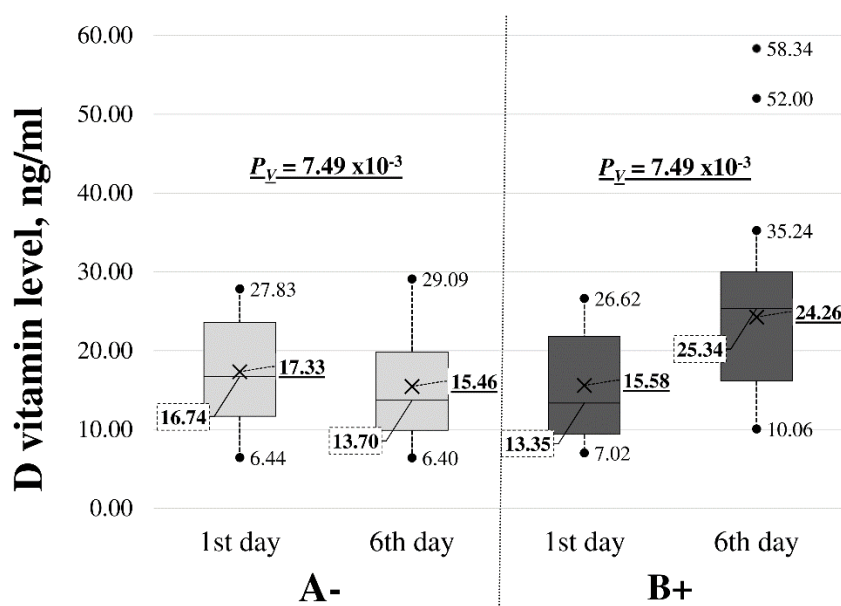


Figure 1. Changes in vitamin D, ng/ml, before and after (Day 1 and Day 6) in groups A - and B +. The graphic shows the median (boxing middle line; value in a dashed square) with quartile distribution in patients. The end of the dashed line outside boxing has a maximum and minimum value; outside the line is expressed in terms of outside the existing value; X – arithmetic mean (underlined); ΔD – the value of vitamin D change in each group; P_v – statistical reliability for paired data with Wilcoxon signed-rank test; P_{mv} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test.

In the placebo group, vitamin D level decreased on average by -1.87 ± 1.97 ng/ml. The resulting mean change was not statistically significant.

Table 3. Changes in vitamin D levels according to case and control group

Group	Delta values				Statistical analysis	
	Mean, ng/ml	SD, ng/ml	Median, ng/ml	IQR	P_{mv}	η
A (placebo)	-1.87	1.97	-1.88	3.05	2.72×10^{-14}	0.44
B (vitamin D)	8.69	7.63	5.82	8.37		

SD – standard deviation; IQR - interquartile range; P_{MV} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test; η - eta or relationship between the nominal and numerical indicators; Value in bold - statistically significant.

There were no statistical differences in delta values of vitamin D level comparing disease-severity groups (Table 4). However, the largest increase in vitamin D level was observed in patients with a severe form of COVID-19. The median value in the severe group was twice as high than in the mild group.

Table 4. Changes in vitamin D levels according to severity of the disease

Severity of COVID-19	Delta values (vitamin D)				Statistical analysis		
	Mean, ng/ml	SD, ng/ml	Median, ng/ml	IQR	$P_{F \text{ or } KV}^*$	$P_{T \text{ or } MV}^{\wedge}$	
A- (placebo)							
Mild	-1.58	2.15	-2.14	3.97	<u>0,43</u>	1.44×10^{-11} Mi: $3,10 \times 10^{-4}$ Mo: $4,31 \times 10^{-8}$ S: $7,96 \times 10^{-4}$	
Moderate	-1.72	1.83	-1.83	3.03			
Severe	-2.93	2.31	-4.08	4.35			
B+ (vitamin D)							
Mild	5.53	3.65	4.06	5.86	0.14		
Moderate	6.57	3.64	5.46	4.14			
Severe	12.44	10.43	7.92	14.51			

SD – standard deviation; IQR - interquartile range;

* - P_F - statistical reliability with ANOVA (underline value) of mean; P_{KV} - statistical reliability with Kruskal–Walli test of median;

\wedge - P_T – statistical reliability of the difference in delta mean between the two groups (A- vs B+) using the T-test (underline value); P_{MV} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test;

Mi - Mild; Mo - Moderate; S - Severe; Value in bold - statistically significant.

Changes in C - reactive protein levels

The decrease of C-reactive protein (CRP) was observed in both groups. However, the delta indicators were not in a normal distribution, thus the medians were compared (Figure 2).

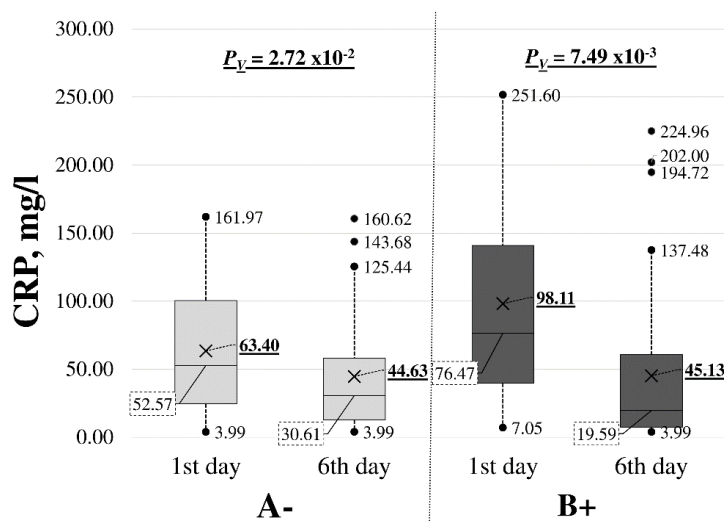


Figure 2. CRP, ng/ml, pre and post changes (Day 1 and Day 6) in groups A - and B +. The graphic shows the median (boxing middle line; value in a dashed square) with quartile distribution in patients. The end of the dashed line outside boxing has a maximum and minimum value; outside the line is expressed in terms of outside the existing value; X – arithmetic mean (underlined); ΔD – the value of vitamin D change or delta in each group; P_v - statistical

reliability for paired data with Wilcoxon signed-rank test; P_{mv} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test.

The mean delta values of CRP level before and after the treatment (Table 5) in the B vial group is significantly higher than in the A vial group: -52.98 ± 82.34 mg/l vs. -18.77 ± 54.17 mg/l. The differences between two groups were statistically significant ($p < 0.05$).

Table 5. Changes in CRP levels comparing case and control group

Group	Delta values				Statistical analysis	
	Mean, ng/ml	SD, ng/ml	Median, ng/ml	IQR	P_{mv}	η
A (placebo)	-18.77	54.17	-15.59	46.85	$4,24 \times 10^{-2}$	0.05
B (vitamin D)	-52.98	82.34	-37.74	87.57		

SD – standard deviation; IQR - interquartile range; P_{mv} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test; η - eta or relationship between the nominal and numerical indicators. Value in bold - statistically significant.

No statistically significant differences in delta values of the CRP level in mild and severe groups were found (table 6). However, the decrease in CRP level in a moderate severe group was statistically significant compared to the placebo group ($p < 0.05$). Therefore, it can be concluded that vitamin D in a moderate-severe COVID-19 patient contributes to the reduction of CRP level.

Table 6. Changes in CRP levels according to severity of the disease

Severity of COVID-19	Delta values (CRP)				Statistical analysis	
	Mean, ng/ml	SD, ng/ml	Median, ng/ml	IQR	P_F	P_T^{\wedge}
A- (placebo)					0.57	0.31 Mi: 0.69 Mo: 4.06×10^{-2} S: 0.82
Mild	-23.69	36.09	-20.77	51.40		
Moderate	-11.81	54.23	-11.02	44.97		
Severe	-40.15	79.70	-19.00	147.03		
B+ (vitamin D)					0.50	
Mild	-32.05	42.62	-20.73	73.75		
Moderate	-64.60	96.72	-64.40	145.62		
Severe	-49.38	79.79	-40.27	66.68		

SD – standard deviation; IQR - interquartile range; P_F - statistical reliability with ANOVA (underline value) of mean;

\wedge - P_T – statistical reliability of the difference in delta mean between the two groups (A- vs B+) using the T-test ; Mi - Mild; Mo - Moderate; S - Severe; Value in bold - statistically significant.

Changes in ferritin levels

The response of other inflammatory markers such as ferritin and IL-6 were also investigated. The ferritin level in the placebo group decreased on average, but in the B group, the indicator increased on average (Table 7). It should be noted that there was no normal distribution in group B thus the median values were compared. Despite the rise in average ferritin value, the median delta value in B group indicated a decrease of ferritin level. The differences between true and placebo groups were not statistically significant, and obtained data also showed a very large distribution in blood levels of ferritin. (supplementary material, table S2-S3)

The ferritin delta values between the two groups were also not statistically significant applying a non-parametric data analysis method. In addition, there was a high dispersion in both groups.

Table 7. Changes in ferritin levels comparing case and control group

Group	Delta values				Statistical analysis	
	Mean, ng/ml	SD, ng/ml	Median, ng/ml	IQR	P_{mv}	η
A (placebo)	-51.51	288.02	-82.80	402.73	0.28	0.03
B (vitamin D)	90.98	493.76	-26.40	469.93		

P_{mv} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test; η - eta or relationship between the nominal and numerical indicators

Changes in IL-6 levels

A decrease of IL-6 was observed in both groups, but the delta value was not in the normal distribution, thus non-parametric data methods were applied. There was no statistically significant difference in delta values between the groups ($P > 0.05$). The wide range of delta values in both groups, especially in group A (Table 8) may have contributed to such results.

A possible explanation of such distribution could be explained by the different baseline levels, which could again be tested by analysis in different groups of patients with disease severity. However there was also no statistically significant difference between the severity groups of patients.

It should be noted that there was one patient in each group who showed significantly higher IL-6 levels compared to others (supplementary materials Tables S4-S5). The IL-6 levels in the patients of A and B groups were 1552.10 pg/ml and 1766.50 pg/ml respectively. Due to this, two patients were excluded from analysis.

Table 8. Changes in IL-6 levels comparing case and control group

Group	Delta values				Statistical analysis	
	Mean, pg/ml	SD, pg/ml	Median, pg/ml	IQR	P_{mv}	η
A (placebo)	-10.99	54.93	-4.20	43.95	0.29	0.01

B (vitamin D)	-17.29	37.18	-20.90	41.60		
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P_{MV} – statistical reliability of the difference in delta medians between the two groups (A- vs B+) using the Mann-Whitney U test; η - eta or relationship between the nominal and numerical indicators

4. DISCUSSION

Our data showed that large doses of vitamin D may effectively decrease CRP level in vitamin D deficient COVID-19 patients during just 5-day-intervention compared to those who haven't received vitamin D. The increase of vitamin D level by average 8 ng/ml in blood was accompanied by decrease of CRP level in patients with moderately severe clinical manifestation of the disease. In the study we have defined the true case group to investigate the effect of the vitamin D level increase on inflammatory markers compared to those, who haven't received the supplement. B group patients whose vitamin D levels did not rise above the coefficient of variation were excluded. It was necessary to analyze the effect of the increase of vitamin D levels on inflammatory markers. The true placebo group was defined to exclude an uncontrolled increase of vitamin D levels from the food supplements or other exogenous sources of vitamin D in A group.

Based on the results, one might assume that only the moderate severity of COVID-19 has the most positive outcome. However, it is worth mentioning that in most cases mild clinical manifestation of the disease is self-limiting, thus the intervention with vitamin D might not cause a statistically significant improvement. The severe form of COVID-19 is not significantly affected by vitamin D, thus showing that the supplementation with vitamin D3 should be investigated according to other comorbidities and drug interactions.

For instance, a similar randomized placebo-controlled study (SHADE) where participants have been randomized to receive 60,000 IU of cholecalciferol daily for 7 days did not show any effect on IL-6, ferritin, and CRP levels [54]. Comparing two studies we have seen the differences in dosing and patients' recruitment criteria. During the SHADE study, the level of vitamin D has been increased by ~40 ng/ml on average, compared to the 8.6 ng/ml increase in our study. Such a change of vitamin D level has been achieved by 60,000 IU/daily intervention, compared to our intervention (12,000 IU/daily). It is worth mentioning that the included population in the SHADE study didn't have a significantly high level of CRP to study the impact of vitamin D. Secondly, the population was significantly lower compared to our study. Thirdly, there was no stratification of patients by disease severity compared to the study provided in our research site. Additional survival study, however, is necessary to verify the most appropriate therapy for severe COVID-19 form.

The total vitamin D dose in our study was at 72,000 IU which was much lower compared to other high-dose interventions [12, 29, 30, 54].

The study with a single oral bolus dosage of 100 000 IU of cholecalciferol did not show any improvement in the outcomes of COVID-19. However, this study shows that a lower D vitamin level at admission time was associated with higher pulmonary involvement and a higher rate of ICU admission. According to this study, the level of 25(OH)D >25 ng/ml was associated with better outcomes [29]. Suggesting this it may be helpful to provide screening blood tests of vitamin D to select patients with a higher risk and start immediate supplementary treatment with cholecalciferol.

Studies with a single high bolus dose of 200,000 IU of cholecalciferol did not show any effect on mortality, length of hospital stay, hospital discharge, ICU admission, and rates of mechanical ventilation, despite achieving sufficient vitamin D levels ≥ 30 ng/mL [12].

However, studies with a larger single dose of 400,000 IU compared to the standard dose of 50,000 IU in older adults within 72 hours after the diagnosis of COVID-19 were associated with reduced overall mortality on day 14. The increase of vitamin D levels on day 7 was significantly higher in the high-dose group on average at 38 ng/ml compared to 8.4 ng/ml in the standard-dose group [30].

There are some trials with smaller population groups receiving smaller doses of cholecalciferol for 2 weeks [31, 32].

In another multicenter randomized controlled trial patients with mild to moderate COVID-19 and suboptimal vitamin D were receiving 5000 IU and 1000 IU of oral cholecalciferol daily for 2 weeks. This study reports that a 5000 IU daily oral vitamin D3 supplementation significantly shortens recovery time to symptoms, but shows no significant differences in ICU, mortality events, and days to discharge. Within-group comparisons also showed a significant decrease in IL-6 levels over time in both groups but were not clinically significant in between-group comparisons. However, the increase in vitamin D level was statistically significant on average at only 3.6 ng/ml. It is worth mentioning that 47% of patients also received vitamin C supplementation [31].

In a small randomized clinical trial, 22 outpatients with a mild disease course received oral supplementation of 10,000 IU daily of vitamin D3 for 14 days. The administered supplementation was sufficient to increase total vitamin D serum levels significantly on day 14 (28.2 ng/mL on average). And the intervention group had fewer symptoms on the 7th and 14th day of follow-up [32]. According to research conducted in France (80,000 IU/2000 g), using vitamin D3 during COVID-19 hospitalization does not lessen the risk of death [33]. Additional studies are necessary to analyze mortality in patient groups who have received vitamin D according to our protocol and to find out the optimal dosage.

We provide such a kind of placebo-controlled prospective study in order to escape several limitations of other studies. E.g., in the case of observational studies the randomization was limited due to clinical judgments of healthcare providers according to patients' clinical condition [10]. In the case of prospective studies, there is a limited amount of placebo-controlled trials. There are limitations in statistical analysis in such experiments, where only the intervention has been taken into account escaping the true changes in blood vitamin D levels, which are the key factor measuring the changes in inflammatory markers [12, 13, 53].

According to the result of this study vitamin D did not produce any significant changes in ferritin and IL-6 levels. Other studies support current findings. A retrospective study by Feld et al., for example, showed that despite greater ferritin levels being related to all-cause mortality, ferritin could not predict numerous critical outcomes, including death, with any certainty [40]. Some researchers suggested that ferritin levels could be used to predict the severity of COVID-19 [34-52]. On the other hand, a retrospective cohort study that included 158 confirmed COVID-19 patients showed that COVID-19 patients with low serum iron levels were prone to suffer from a severe disease and multiple-organ damage [52]. In our case, IL-6 levels, similarly to ferritin levels, did not show any statistically significant changes. A supporting study by Fernandes et al indicated that a single high dose of 200 000 IU vitamin D had no effect on cytokines, chemokines, and growth factors in patients with moderate to severe COVID-19 [53].

5. Limitations

This study did not question the impact of medications (dexamethasone and remdesivir) on the treatment outcomes.

6. Conclusions

There was a rapid elevation of circulating 25-hydroxyvitamin D levels in hospitalized vitamin D deficient COVID-19 patients treated by high-dose sublingual sprayable vitamin D microemulsion for five days. High-dose vitamin D therapy was accompanied by significant decrease in CRP levels in patients with a moderate to severe illness. We were not able to detect statistically significant changes in ferritin and IL-6 levels during the short term high-dose vitamin D spray therapy. Additional studies must be done to verify the correlation between vitamin D intervention and mortality outcomes.

List of abbreviations

COVID-19 - Coronavirus disease 2019

25(OH)D - 25-hydroxyvitamin D

ARVI - Acute Respiratory Tract Viral Infections

CRP - C-Reactive Protein

IL-6 - Interleukin 6

SD - Standard deviation

IQR -interquartile range

cv - coefficient of variation

P_{KV} - statistical reliability of Kruskal-Wallis test

P_F - statistical reliability of ANOVA test

P_{MV} - statistical reliability of Mann-Whitney test

P_T - statistical reliability of T-test (statistical confidence)

BMI - Body Mass Index

GFR Glomerular Filtration Rate

PCR - Polymerase Chain Reaction

PSCUH - Pauls Stradiņš Clinical University Hospital

ICU - Intensive Case Unit

MR - magnetic resonance

Declarations

Ethics approval and consent to participate:

The study has been conducted according to the guidelines of the Declaration of Helsinki, and has been evaluated and received permission from the local Ethics committee of the Pauls Stradiņš Clinical University Hospital (Approval number: 170621-24L).

Consent for publication

We grant exclusive rights on royalty-free basis to Multidisciplinary Digital Publishing Institute ("MDPI") its licensees and where the relevant Journal is co-owned by MDPI to the co-owners of the Journal, to publish the Work in *Nutrients* and to exploit all rights.

Availability of data and materials

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

No competing interests.

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