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The Effects of 10,000 IU Vitamin D Supplementation on Improvement of Clinical Outcomes, Inflammatory and Coagulation Markers in Moderate COVID-19 Patients: A Randomized-Controlled Trial

Nurpudji ASTUTI TASLIM[1], Zahratul FAJRI[2], Sisca Agustia OLII[2], AMIRAH[2], Mardiana MADJID[1,3], A Yasmin SYAUKI[1,3], Irawaty DJAHARUDDIN[3,4], Agussalim BUKHARI[1,3], Suryani AS'AD[1,3], Haerani RASYID[1,5]

1 Department of Nutritional Science, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
2 Clinical Nutrition Specialist Program, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
3 Wahidin Soedirohusodo Hospital, Makassar, Indonesia.
4 Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
5 Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

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ABSTRACT

Background & aims: Vitamin D supplementation as an immunomodulator has been identified as a potential strategy to prevent and treat Coronavirus disease 2019 (COVID-19). We aimed to analyze the effect of 10,000 IU vitamin D3 supplementation on 25(OH)D levels on primary clinical outcomes (conversion length), inflammatory markers (Total Lymphocyte Count (TLC), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR)) and coagulation marker (D-Dimer) in moderate COVID-19 patients at Wahidin Sudirohusodo Hospital, Makassar, Indonesia.

Methods: We conducted a single-blind randomized-controlled trial on the confirmed moderate COVID-19 patients above 18 years old and low vitamin D status. Each of intervention and control groups were supplemented of 10,000 IU and 1000 IU cholecalciferol that taken daily for 2 weeks. Levels of 25(OH)D were analyzed for the primary endpoint (conversion length), then correlated to secondary endpoints (Length of Stay (LOS)), clinical manifestations improvement, and markers TLC, NLR, PLR, and D-Dimer serum, handgrip strength (HGS) as functional capacity measurement, after adjusted to age, sex, nutritional status based on body mass index (BMI) and *Subjective Global Assessment* (SGA) tool, comorbidities, and anti-coagulant administration. Medical nutritional therapy was given and presented as energy, protein, carbohydrate, and fat achievement, and vitamin D intake was also calculated.

Results: A significant effects was found in 60 samples with pre-intervention vitamin D deficiency (61.7%) and insufficiency (38.3%) status, and 10,000 IU of vitamin D3 supplementation could increase 25(OH)D levels within 2 weeks to reach sufficiency status (16.7%). The Vitamin D3 supplementation of 10,000 IU and 1000 IU could significantly increase 25(OH)D levels compared to the control group of 1000 IU (4.61±5.43 vs. -0.29±2.72; P <0.0001) and it was correlated to primary clinical outcome, which is length of conversion (6.53±1.17 vs 10.47±2.56; P < 0.0001). The increase in HGS (6.61±3.01 vs. 4.04±4.44; P = 0.011), LOS (11.63±2.5 vs. 14.73±3.45; P = 0.001), and improvement in clinical manifestations were found to be significant in both groups. We analyzed changes the effect of vitamin D supplementation in TLC, NLR, and D-Dimer as marker of coagulopathy associated COVID-19 on both groups that showed were not significant. Positive and significant correlation was only showed on PLR levels after intervention (r=0.368; P=0.045).

Conclusion: Supplementation of vitamin D3 10,000 IU in moderate COVID-19 patients had a significant effect on 25(OH)D level, length of conversion, LOS, functional capacity, and PLR levels, but it has negative correlation in TLC, NLR, and D-Dimer levels.

Correspondencia:
Nurpudji Astuti Taslim
pudji_taslim@yahoo.com

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KEYWORDS

COVID-19, Vitamin D, Clinical Outcome, Nutritional Status, Inflammatory markers, and Coagulopathy.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a worldwide pandemic that caused by coronavirus 2 (SARS-CoV-2) and mostly represented as bilateral interstitial pneumonia, the virus enters the respiratory tract via the angiotensin-converting enzyme 2 (ACE-2) receptor on type II pneumocytes and many organs[1]. Administration of vitamin D has been identified as a potential strategy to prevent or treat COVID-19. A recent meta-analysis concluded that the vast majority of COVID-19 patients suffer from vitamin D deficiency and shown a positive association to COVID-19 severity and mortality[2].

COVID-19 is a systemic infectious disease that can affect the function of hematopoiesis, hemostasis, and immunity. Low lymphocyte counts are associated with severe COVID-19 and mortality[3]. The results of a complete blood count in COVID-19 showed lymphopenia (83.2%), thrombocytopenia (36.2%) and leukopenia (33.7%)[4]. A low Total Lymphocyte Count (TLC) serves as a marker for rapid identification of COVID-19 patients with more severe clinical presentations[3]. In addition to TLC, an increase in the Neutrophil-to-Lymphocyte Ratio (NLR) can be used as an alarm and is recommended to assess the prognosis, evaluate the degree of disease based on the clinical symptoms of the patient, and determine the appropriate treatment for COVID-19 patients[5].

Vitamin D has an influence on COVID-19 through several mechanisms, one of which is through the induction of cathelicidin and defensins which are able to reduce the rate of virus replication and reduce the concentration of pro-inflammatory cytokines. Inflammatory markers in COVID-19 that are used as other prognostic predictors are dynamic changes in platelet count and lymphopenia. Platelet-to-Lymphocyte Ratio (PLR) was higher in the length of conversion (LOC) and length of stay (LOS) due to COVID-19 and the PLR changes were more prominent in critically ill patients that caused by a cytokine storm that triggers inflammation resulting in the stimulation and release of platelets. Higher PLR was seen in severe patients (436.5±329.2) compared to mild-moderate patients (176.7±84.2; p<0.001)[6].

A number of studies have also found the incidence of coagulopathy in patients with COVID-19. Abnormalities in coagulation parameters (D-Dimer) and poor prognosis were found in 183 COVID-19 patients. Fibrinogen concentrations and antithrombin activity appear to decrease as the disease progresses[7]. PLR and D-Dimer have been evaluated as markers of inflammation and coagulation to predict the severity of COVID-19. Several studies have shown the role of vitamin D in inhibiting the process of vascular thrombosis in endothelial cells and reducing hyperinflammation. RCT that was conducted in Brazil by given single oral dose of 200,000 IU of vitamin D3 (cholecalciferol) supplementation in moderate-to-severe COVID-19 patients was showed a non-significant LOS compared to the placebo group (P = 0.62), but significant changes in 25(OH)D levels (p<0.001)[8]. While research in India, daily vitamin D3 supplementation of 60,000 IU helps achieve 25(OH)D levels >50 ng/ml in 75% of COVID-19 patients on day 14 and SARS-CoV-2 RNA was negative by day 21 and was considered significant in the clearance of SARS-CoV-2 (p<0.001), but not significant for D-Dimer levels and other inflammatory markers (P = 0.241)[9]. A similar study in Saudi Arabia between dose of vitamin D3 5000 IU vs 1000 IU administered for 2 weeks in mild-moderate COVID-19 patients showed a shorter LOC (6.2±0.8 vs. 9.1±0.8; P = 0.039) and a significant effect on D-Dimer levels (P = 0.02), but not significant on 25(OH)D levels pre-post intervention (P = 0.67)[10].

There have been no results of randomized clinical trials of certain doses of vitamin D on clinical outcomes, markers of inflammation and coagulation of COVID-19 patients in Indonesia. This study aims to determine the effect of vitamin D3 supplementation with a dose of 10,000 IU vs 1000 IU on LOC, LOS, and improvement of clinical manifestations, functional capacity, nutritional status, as well as analyzing the correlation of 25(OH)D levels with TLC, NLR, PLR and D-Dimer in moderate COVID-19 patients.

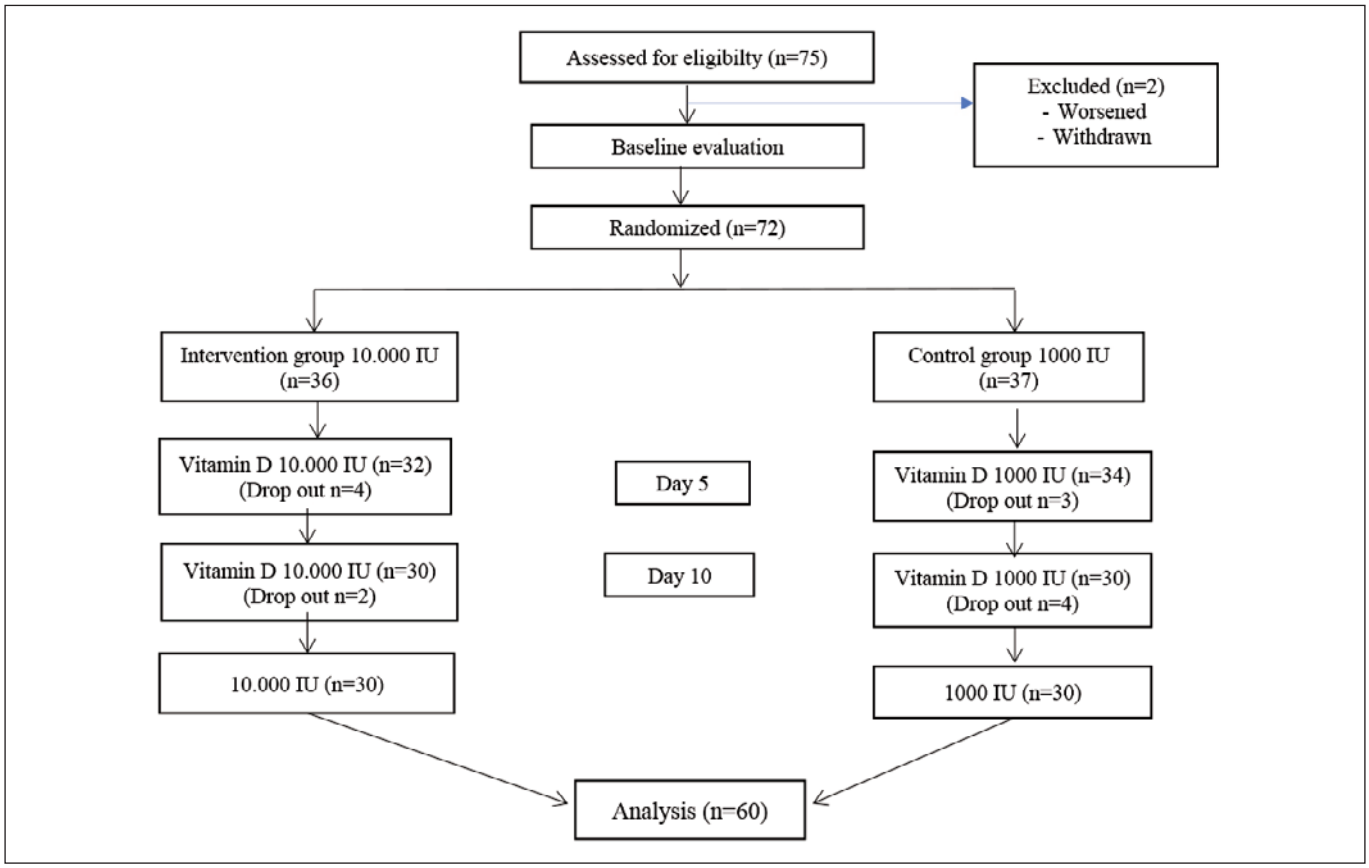
MATERIALS AND METHODS

Study Design and Participants

This study was a single-blind randomized clinical trial that has been carried out at the COVID-19 isolation ward in Wahidin Sudirohusodo Hospital, Makassar, Indonesia, from April 1st to September 30th, 2021 (Figure 1). The population of this study were confirmed COVID-19 patients through the nasopharyngeal swab examination with Reverse Transciptase-Polymerase Chain Reaction (RT-PCR), as evidenced by the Cycle Threshold (CT) value <40, who met the inclusion criteria: moderate confirmed COVID-19 with clinical signs of pneumonia (fever, cough, shortness of breath, rapid breathing) but no signs of severe pneumonia including SpO2 > 93% with room air, aged > 18 years; informed consent; and have vitamin D insufficiency or deficiency status (< 30 ng/dL)[11].

The exclusion criteria were pregnant or breastfeeding confirmed COVID-19 patients; had an acute or chronic disease that was being treated with certain medications, such as chronic kidney disease (CKD) patients undergoing hemodialysis, patients infected with human immunodeficiency virus (HIV) and receiving antiretroviral (ARV) drugs, tuberculosis patients receiving anti-TB drugs; patients with malignancies undergoing

Figure 1. CONSORT Flow Diagram



chemotherapy or radiotherapy, or leukemia or malignant lymphomas receiving routine transfusions; had received Vitamin D3 Supplementation > 1000 IU/day for > 3 days; Creatinine level > 2.0 mg/dL; Previously treated with mechanical ventilation; Nasopharyngeal RT-PCR swab results were negative after being given Vitamin D3 < 5 days; Experiencing worsening of symptoms, such as oxygen saturation <93%, decreased consciousness, required oxygen support in the form of High-Flow Nasal Cannula (HFNC)/Extracorporeal membrane Oxygenation (ECMO) via a ventilator, or died; Hypersensitivity to Vitamin D3 10,000 IU; and refused to take blood samples after negative conversion for post intervention.

This study has received approval from the research ethics committee of the Medical Faculty of Hasanuddin University with the number B26/UN4.6.4.5.31/PP36/2020, and the protocol number 0411212204. This study has also been registered in the ClinicalTrial.gov database with the identification number NCT05126602.

Randomization

The randomization process in this study was carried out in a simple random sampling technique by assessing the patients and receiving vitamin D supplementation of 10,000 IU and 1000 IU alternately (Figure 1). Each sample was given an explanation and asked for approval to participate in the study by signing the informed consent form and witnessed by 2 people (Researcher and Nurse who served in the redzone), then documented. Blinded in the research conducted on participants, investigators, and outcome assessors.

Study Protocol

Vitamin D3 Supplementation (Cholecalciferol) was given to the intervention group with the Hi-D® brand given orally at a dose of 5000 IU twice a day for 2 weeks (10,000 IU/day) every 08.00 and 20.00 WITA., while the control group was given Hi-D® 1000 IU/day every 08.00 WITA. The study was conducted for 2 weeks. The form of vitamin D3 given was white, round, 0.3 mm in size, is a chewable tablet, with a chocovanilla taste. Circular permit from BPOM: DKL 1909504363A1.

Data Collection

Data collection were screening for sampling and data collection from all variables, including anthropometric measurements and handgrip strength using a dynamometer, then measured hematologies parameter pre-post intervention, there were routine blood, D-Dimer, and levels of 25(OH)D which were analyzed using an ELISA kit manufactured by

Shanghai China, Thermo brand, with Bioassay Technology reagent catalog number E-EL-H1343 at wavelength of 450 nm in the Teaching Hospital Laboratory (RSP) Hasanuddin University. We calculated the energy, macronutrient composition, and the vitamin D intake by Nutrisurvey® application after giving the medical nutritional therapy for 2 weeks during intervention.

Data Analysis and Sample Size Calculation

The data obtained were processed and analyzed statistically using the SPSS 25 for Windows method and the results were displayed in the form of narration and tables. The level of confidence was 95% and considered significant if $p < 0.05$. T-test compared numerical data using two categories or two groups using independent t test if the data was normal, Mann Whitney test if it was not normal. To compare numerical data in repeated measurements of two observations (pre-post) used t-paired test if the data were normal, and the Wilcoxon test if it was not normal. To test the correlation using the Spearman test if the data was not normal, and the Pearson test if it was normal.

Based on a study in Saudi Arabia, the sample size looked at the duration of negative conversion where the group receiving 5000 IU had an average conversion duration of 6.2±0.8[10]. This RCT study was expected to shorten LOC by up to 10%. Determination of the sample size using the difference between the two means formula, the effect size obtained from this result is 0.775, with 95% Confidence Interval (CI), then the number needed for each group was 30 patients.

RESULTS

Baseline Characteristics of Participants

This study involved 60 patients with moderately confirmed COVID-19 with the following characteristics: The mean age in the group was 10,000 IU compared to 1000 IU (37.47±11.61 vs. 41.43±15.41; P = 0.304). Gender was dominated by 32 men (53.3%). Based on nutritional status assessment using BMI, most patients were overweight and underweight BMI, respectively 36.7%, meanwhile based on SGA, it dominated by moderate protein energy malnutrition (score B); P = 0.792. Medical nutritional therapy was given in both grup. See Table 1.

Table 1. Characteristics of the Research Sample

Variable	10,000 IU	1000 IU	p-value
	30	30	
	n (%), mean±SD	n (%), mean±SD	
Age (years)	37.47±11.6	41.43±15.4	0.304**
Gender (M/F)	12/18	20/10	0.070*
Nutritional Status (n; %)			
BMI (kg/m2)	22.58±4.23	22.26±4.94	0.935**
Obesity (8; 13.3)	6 (20)	2 (6.7)	0.247*
Overweight (22; 36.7)	8 (26.7)	14 (46.7)	
Normal (8; 13,3)	5 (16.7)	3 (10)	
Underweight (22; 36.7)	11 (36.7)	11 (36.7)	
SGA (B/C)	11/19	17/13	0.792*
Nutritional Intake			
Energy (kcal)	1648,71±274,42	1630,12±326,50	0.564**
Protein (g)	69,62±24,57	68,83±19,62	0.762**
Carbohydrate (g)	236,40±62,79	215,82±57,11	0.139**
Fat (g)	66,57±26,36	53,56±24,42	0.015**
Vitamin D (µg)	7,15±2,48	7,06±2,54	0.890*

* Independent t-test; ** Mann Whitney test.

Table 1 continuación. Characteristics of the Research Sample

Variable	10,000 IU	1000 IU	p-value
	30	30	
	n (%), mean±SD	n (%), mean±SD	
Comorbidity (n; %)			
Hypertension (12; 20)	7 (23.3)	5 (16.7)	0.461*
Type II DM (9; 15)	5 (16.7)	4 (13.3)	
Cardiovascular Disease (9; 15)	4 (13.3)	5 (16.7)	
Obesity (8; 13.3)	6 (20)	2 (6.7)	
Malignancy (7; 11.7)	4 (13.3)	3 (10)	
Infectious Diseases (6; 10)	2 (6.7)	4 (13.3)	
Other Diseases (5; 8.3)	1 (3,3)	4 (13.3)	
No Comorbid (4; 6,7)	2 (6.7)	2 (6.7)	
Pre-Intervention Vitamin D Status (n; %)			1.00*
Deficiency (37; 61.7)	19 (63.3)	18 (60)	
Insufficiency (23; 38.3)	11 (36.7)	12 (40)	
Post-Intervention Vitamin D Status (n; %)			
Deficiency (27; 45)	9 (30)	18 (60)	0.03*
Insufficiency (23; 38.3)	13 (43.3)	10 (33.3)	
Sufficiency (10; 16.7)	8 (26.7)	2 (6.7)	
CT value	29,645±5.62	30.87±5.16	0.403**
Use of Anticoagulants	13 (43.3)	18 (60)	0.301*
Coagulopathy	18 (60)	29 (96.7)	0.002*

* Independent t-test; ** Mann Whitney test.

Vitamin D status pre-intervention was found to be deficiency (61.7%) and insufficiency (38.3%), while post-intervention, the sufficiency was only achieved 16.7%, P = 0.03. Low CT scores were not associated with 25(OH)D levels at pre-intervention. Comorbidity has a significant effect on the severity of COVID-19 and is influenced by 25(OH)D levels and were dominated by hypertension (20%), use of anticoagulants (43.3% vs. 60%; P = 0.301) and coagulopathy associated COVID-19 (60% vs. 96.7%; P = 0.002). See Table 1.

Primary Endpoint

LOC was the primary clinical outcome in this study and expected that vitamin D3 supplementation in COVID-19 patients at a dose of 10,000 IU can shorten the duration of negative conversion, especially at moderate levels. Dominant COVID-19 patients have comorbidities and low 25(OH)D levels which can prolong hospital stay. In this study, COVID-19 patients with low vitamin D status showed a significant effect on the shorter negative LOC in the 10,000 IU group (6.53±1.17 vs 10.47±2.56; P <0.0001), as well as shortened LOS in hospital (11.63±2.5 vs. 14.73±3.45; P = 0.001). The relationship between both group and LOC through the Spearman correlation test was found to be negative (r = -0.175; P = 0.355), which means that the higher 25(OH)D level in COVID-19 patients, the shorter LOC as well as LOS (r = -0.469; P = 0.009).

Clinical manifestations in the 10,000 UI group of COVID-19 patients showed improvement, especially symptoms of shortness of breath (P=0.011) and nausea (P=0.045) (Figure 2a). There was improvement of using O2 support in

both pre-intervention groups (P=0.019) and oxygen saturation (P=0.026) (Figure 2b and Figure 2c). Other respiratory, gastrointestinal, musculoskeletal, and neurological symptoms were also found to improve, but not significantly, whereas when the conversion was negative, the patient still had residual symptoms.

Secondary Endpoint

25(OH)D levels in both pre-intervention groups (19,15±5.97 vs 18.85±6.24; P 0.848). The intervention of 10,000 IU of vitamin D3 supplementation for 2 weeks can significantly increase 25(OH)D levels (23,76±8.14 vs. 18.56±6.39; P 0.01), with levels of 25(OH)D (4.61±5.43 vs. -0.29±2.72; P <0.0001). In addition, 25(OH)D levels were also found to have a weak positive correlation with CT values (29.65±5.62 vs 30.87±5.16; P=0.403) although not significant (r = 0.142, P = 0.281), meaning that if the level of 25(OH)D increases, the CT also increases. Functional capacity was carried out by measuring handgrip strength using the Wilcoxon test and found significant results in both post-intervention groups (6.61±3.01 vs. 4.04±4.44; P = 0.011). To assess the relationship of vitamin D3 supplementation to HGS using the Pearson statistical test, a positive correlation was found, which indicates that when the 25(OH)D level increases, HGS will also be stronger (r = 0.473, P = 0.008).

To assess the relationship of vitamin D supplementation to 25(OH)D levels and TLC using the Wilcoxon test, a significant relationship was obtained in the group that received 10,000 IU, but the correlation that tested with Spearman test showed no significant results. The analysis of NLR levels through the Mann Whitney test was not significant (P = 0.261), and the Spearman correlation test also showed not significant (P = 0.217 vs P = 0.469). To assess the relationship between NLR, LOC, and LOS at both groups also found not significant (P=0.894). See Table 2 and 3.

The Wilcoxon test was conducted to assess the relationship between vitamin D supplementation and levels of 25(OH)D, PLR and D-Dimer, and a significant relationship was obtained in the group receiving 10,000 IU of vitamin D3 supplementation with the improvement of vitamin D status. PLR levels as a marker of inflammation and coagulation, after supplementing with vitamin D3 and tested with Spearman correlation, the results are significant. However, D-Dimer as a coagulation marker which was analyzed in both groups was not significant (-1.85±2.73 vs -1.30±1.98; P = 0.228).

Figure 2a. Clinical Manifestation based on symptoms

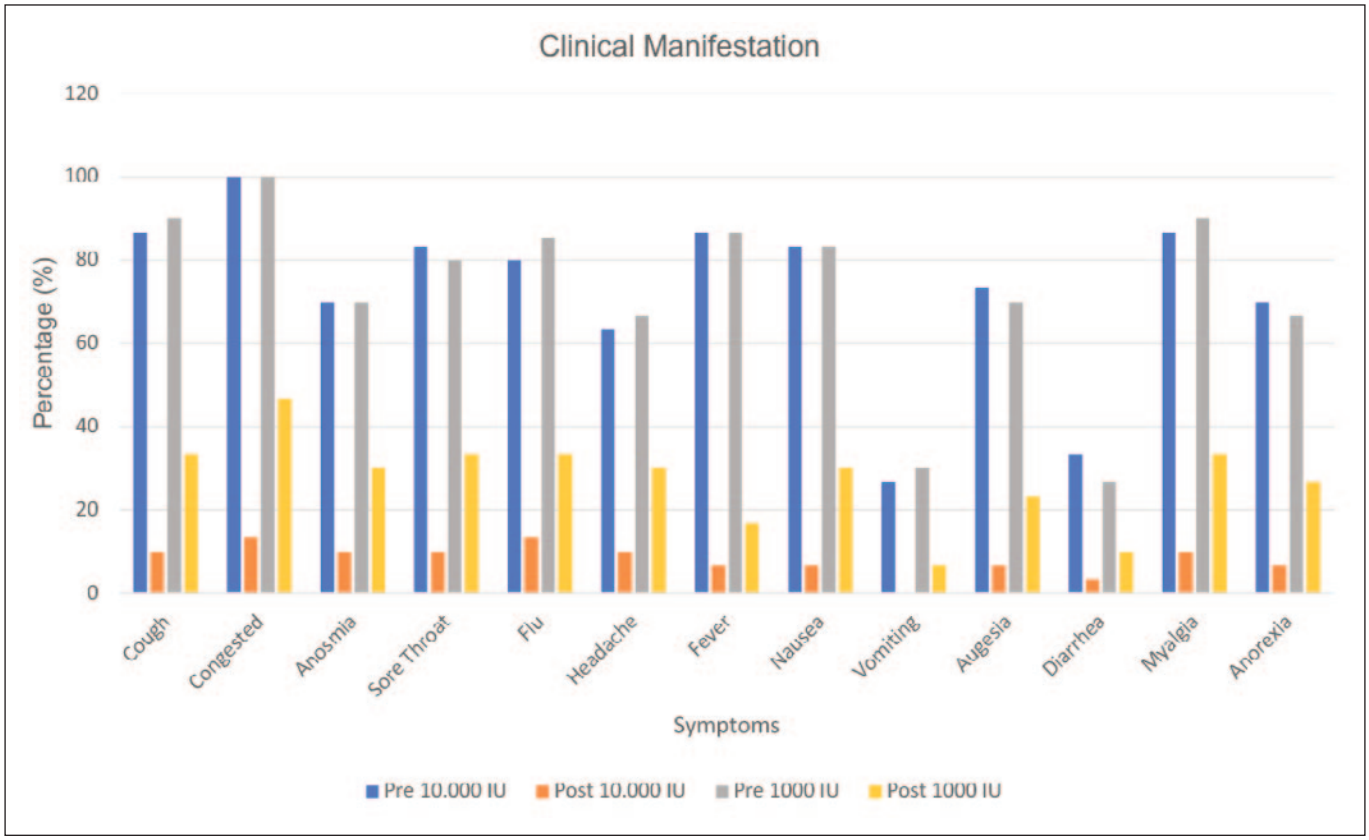


Figure 2b. Percentage of oxygen saturation before and after intervention of vitamin D

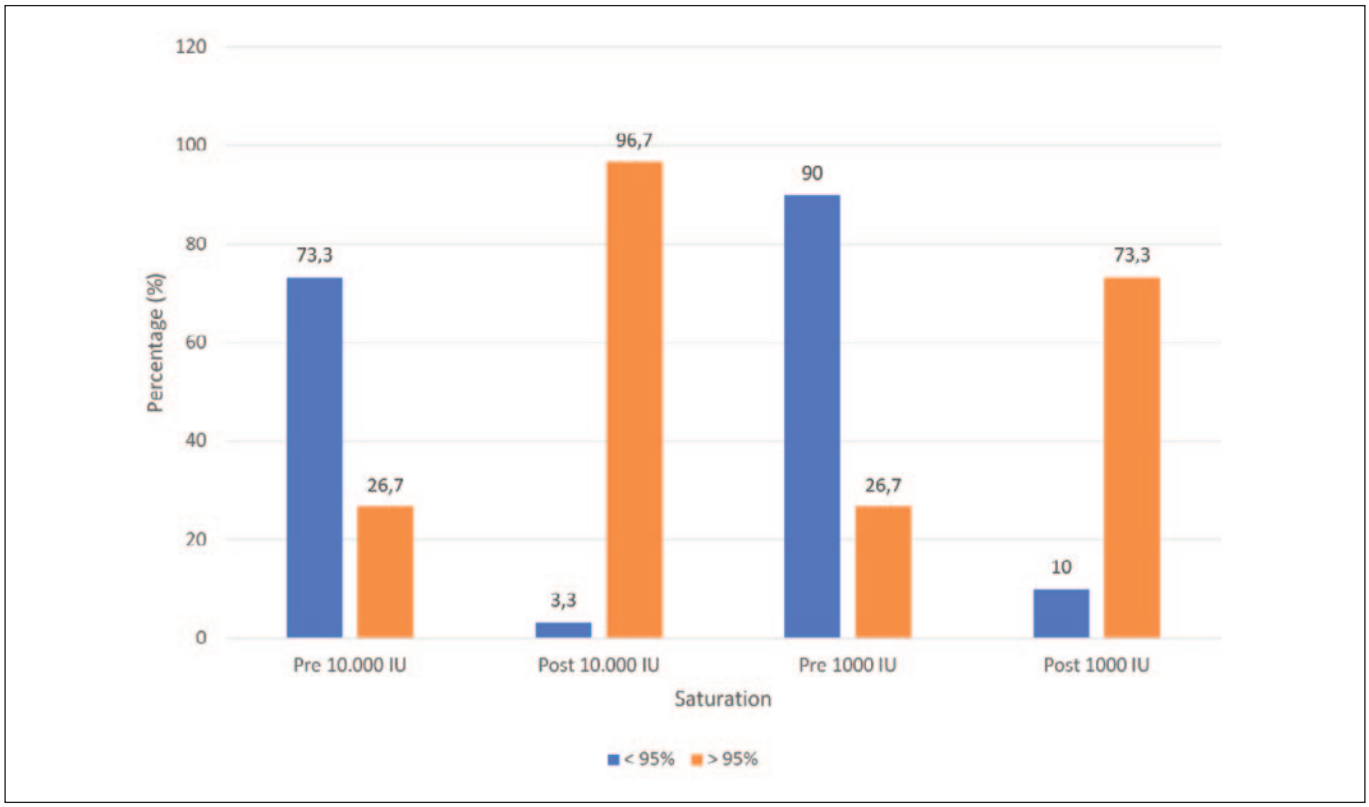


Figure 2c. Oxygen support based on oxygen modality

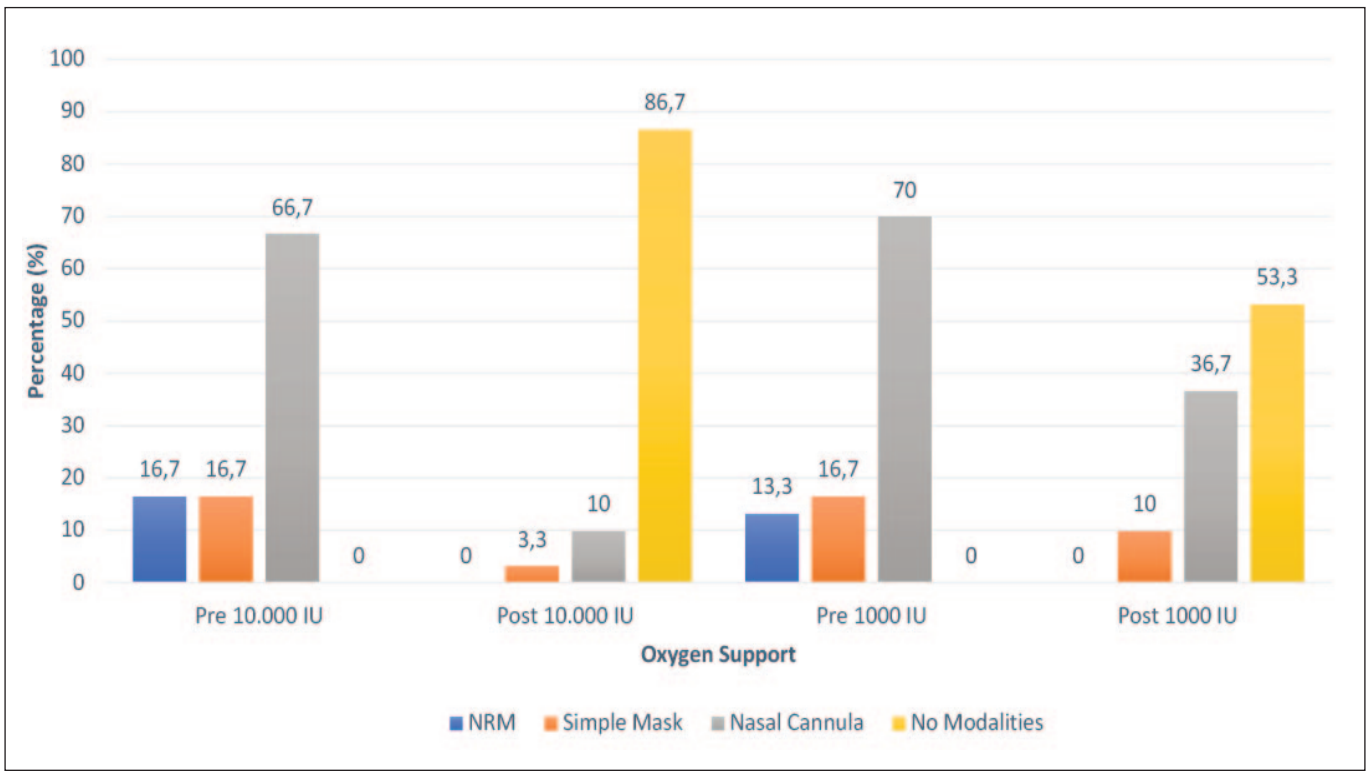
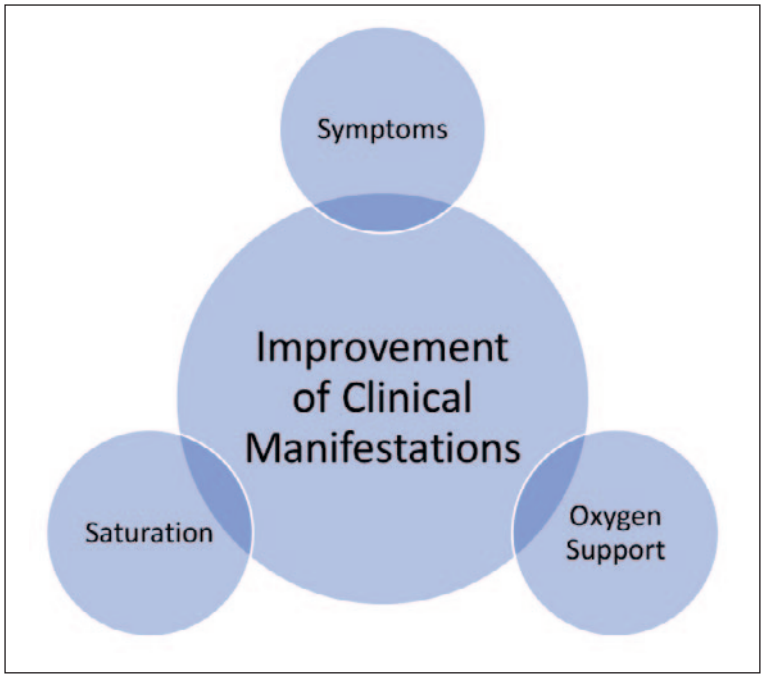


Figure 3. Relationship of Vitamin D Supplementation to Improvement of Clinical Manifestations of Moderate COVID-19 Patients



4. DISCUSSION

Based on the results, 60 moderate COVID-19 patients who met the inclusion criteria, the mean age was 37.47±11.61 vs. 41.43±15.41 (P = 0.304), and gender was dominated by men (53.3%). The incidence of age and gender in COVID-19 patients still varies, a retrospective cohort study of COVID-19 patients conducted by Liu et al (2020) regarding Clinical outcomes of COVID-19 in Wuhan, China, showed that the mean age was around 57 years (47.67) with the most gender being male at 53.4% [12], while an observational study conducted in Europe by Lechien et al on Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019, showed that the average age of hospitalized COVID-19 patients was 39.17±12.09, with the most gender being female at 67.7% [13].

Sex-related biological data are important for investigating the contribution of sex hormones to sex differences in the inflammatory response. The presence of decreased testosterone levels in aging men has been associated with increased levels of proinflammatory cytokines, which may contribute to the worse development of COVID-19 in older men. The sex differences in disease progression could also be attributed to the estrogen-induced decreased expression of ACE-2, which acts as a functional receptor for SARS-CoV-2 to enter host target cells. However, higher stress factors are generally more vulnerable in women, which can be a gender bias in susceptibility to COVID-19 infection[14]. Older age and comorbidities are associated with insufficient intake of vitamin D. Over the age of 60 years, the synthesis of vitamin D in the skin decreases and increases with age due to a decrease in the precursor of vitamin D, 7-dehydrocholesterol in the skin, by about 50% of the age of 20 up to 80 years[15].

Based on nutritional status assessment using BMI, most patients were overweight and underweight BMI, respectively 36.7%, followed by obesity (13.3%), while the SGA was dominated by moderate protein energy malnutrition (score B). Obesity (BMI > 30 kg/m^2) is often associated with low plasma 25(OH)D, making it an age-independent risk factor for COVID-19[16]. Low plasma 25(OH)D values have also been

Table 2. Relationship of Vitamin D Supplementation to Outcomes and Clinical Parameters of Pre-Post Intervention

Parameter	10,000 IU	1000 IU	P Value
Conversion Time	6.53±1.17	10.47±2.56	0.000*
LOS	11.63±2.5	14.73±3.45	0.001*
25(OH)D Level	3.35 (0.93/7.95)	-0.10 (-1.80/0.93)	0.000*
Handgrip Strength	6.61±3.01	4.04±4.44	0.011**
NLR	-1.45 (-2.75/-0.22)	-0.85 (-2.20/-0.05)	0.261*
PLR	-50.36±77.32	-25.02±112.32	0.313**
D-Dimer	-0.80 (-2.37/-0.20)	-0.37 (-1.62/-0.12)	0.228*
TLC	332.94±553.28	170.91±561.48	0.265**

*Mann Whitney test; **Wilcoxon test.

Table 3. Relationship of Vitamin D Supplementation to Inflammation Markers and Coagulation in Moderate COVID-19 Patients

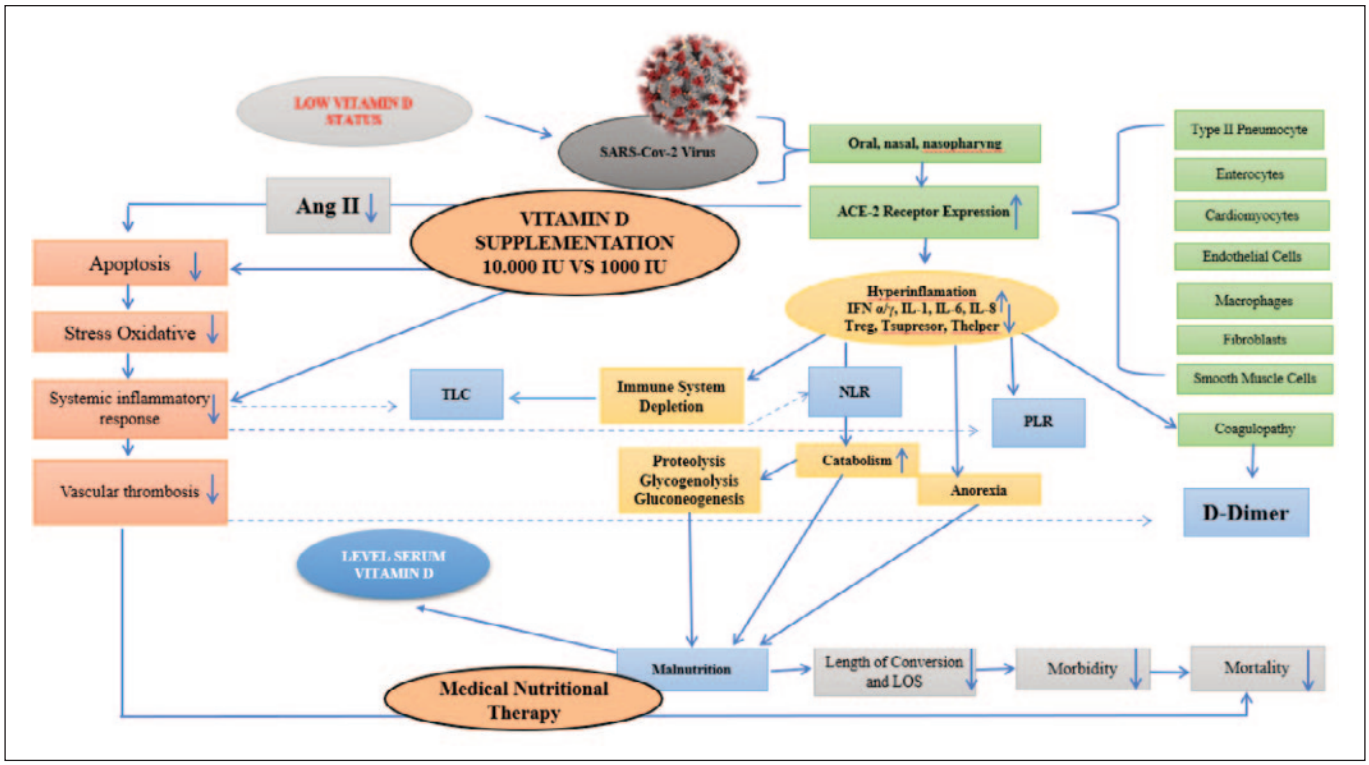
Variable		10,000 IU	1000 IU	p Value
Vitamin D	Pre	19.15 ± 5.97	18.85 ± 6.24	0.848**
	Post	21.60 (8.40 - 36.60)	18.15 (6.80 - 33.50)	
	p Value	0.000*	0.564*	
Handgrip Strength	Pre	6.54 ± 2.97	6.36±2.42	0.798**
	Post	13.15±4.66	10.41±4.43	
	p Value	0.000***	0.000***	
TLC	Pre	1347.40 (642.60 - 3945.00)	1332.00 (554.40 - 3465.00)	0.549**
	Post	1849.90 (979.80 - 3945.00)	1508.40 (866.80 - 3046.00)	
	p Value	0.004***	0.090***	
NLR	Pre	4.10 (0.80 - 12.70)	4.40 (1.07 - 14.00)	0.641**
	Post	2.45 (0.90 - 7.60)	3.40 (1.04 - 12.90)	
	p Value	0.000***	0.001***	
PLR	Pre	175.10 (74.70 - 542.00)	232.08 (79.50 - 584.10)	0.209**
	Post	140.00 (55.50 - 374.30)	193.30 (34.70 - 485.70)	
	p Value	0.000***	0.339***	
D-Dimer	Pre	0.65 (0.19 - 14.20)	1.77 (0.45 - 14.80)	0.039**
	Post	0.40 (0.11 - 6.63)	0.83 (0.27 - 5.00)	
	p Value	0.000***	0.000***	

found in type II diabetes which are associated with an increased risk of metabolic syndrome, hypertension and cardiovascular disease. Insulin resistance associated with low levels of vitamin D[17].

Research conducted by Gang Li *et al* in Wuhan, China in 2020, it was found that the BMI of COVID-19 patients was in the normal range of 22.8±2.9[18], while research in Europe by Roth et al in 2021, obtained a BMI of 30.8±8.5[17]. Study in Spain found that 63% of COVID-19 patients had an SGA score B[19]. The association of malnutrition among patients hospitalized for infection caused by SARS-CoV-2 supported by evidence regarding the nutritional status and prognosis of COVID-19. Underweight adults with respiratory viral infections have a four times higher risk of being hospitalized compared to adults with normal weight. The study of Nicolau et al. found that LOS among patients with severe malnutrition was significantly higher than among those who were not malnourished (18.4±15.6 vs 8.5±7.7 days; p<0.001)[19].

Comorbidity has a significant effect on the severity of COVID-19 and is influenced by 25(OH)D levels. Comorbid diseases are known to influence the course of illness in COVID-19 patients. Several conditions known as comorbid factors in COVID-19 include geriatric, hypertension, heart disorders, obesity, ARDS and DM (Figure 4). In the study of Fiorindi et al (2021), comorbidities in COVID-19 patients in Italy were dominated by hypertension (48%) followed by DM (14.8%), in line with the research conducted by Frasinette (2022) that shown the main underlying comorbid was heart disease (23,5%)[20,21]. Based on a meta-analysis of 30 studies with 53,000 COVID-19 patients, comorbidity is a risk factor for disease severity, it show an association with Renin Angiotensin-Aldosterone-System (RAS), vitamin D status and COVID-19 infection[22]. Low vitamin D status is suspected of contributing to increased RAS activity and high blood pressure. Vitamin D has multiple functions in the cardiovascular system by exerting a protective effect on the endothelium, vascular muscle, and cardiac muscle cells. In a meta-analysis with 65,994 participants, there was a negative

Figure 4. The Effects of 10,000 IU Vs. 1000 IU Vitamin D Supplementation and supported by medical nutritional theraphy in improving TLC, NLR, PLR, D-Dimer and clinical outcomes in COVID-19 Patients



correlation between 25(OH)D vitamin D plasma levels (below 60 nmol/L) and cardiovascular events. Vitamin D supplementation had a positive effect on the respiratory system and cardiovascular system in participants who previously had a vitamin D deficit[23].

The intervention of 10,000 IU of vitamin D3 supplementation for 2 weeks could significantly increase 25(OH)D levels compared to the 1000 IU control group (4.61±5.43 vs. -0.29±2.72; P <0.0001). In the last decade, several studies have shown an association between vitamin deficiency and various diseases including systemic infections. Vitamin D deficiency affects immune function because vitamin D is an immunomodulator, enhancing innate immunity by secreting antiviral peptides through mucosal defenses (Figure 4). A meta-analysis combining data from 8 observations reported that subjects with serum vitamin D concentrations <50 nmol/l (<20 ng/ml) had a 64% increased risk of CAP[16]. Vitamin D deficiency may compromise respiratory tract immune function, increasing the risk of COVID-19 severity and death[6].

In this study, a correlation test was conducted between vitamin D levels in COVID-19 patients before the intervention. Levels of 25(OH)D had a positive correlation with CT values (29.6±5.62 vs 30.87±5.16; P=0.4) through the spearman test although not significant (r = 0.142, P = 0.281). It was also found by a similar study that the proportion of confirmed COVID-19 patients with vitamin D deficiency was higher in the ICU group (82.0 vs. 65.2%). Among these ICU patients, lower vitamin D levels (<50 nmol/L) were associated with younger age (57 vs 67) years, P=0.04) and lower CT values[24].

The primary clinical outcome assessed in this study was conversion time. COVID-19 patients with vitamin D deficiency and insufficiency showed a significant effect on the shorter negative conversion duration in the 10,000 IU group (P <0.0001). Based on a study in Saudi Arabia, the sample size looked at the duration of negative conversion where the group receiving 5000 IU had an average conversion duration of 6.2±0.8[10]. This RCT study is expected to shorten LOC by up to 10%. Yue et al, comparing the length of conversion on the degree of severe and critical illness, found no significant difference between the two. To date, there has been little research on predictors of time for negative conversion of SARS-CoV-2 RNA. Viral shedding has been associated with infectivity and transmission in influenza virus infection, and understanding this is critical for the implementation of prevention strategies. Therefore, it is important to determine the duration of viral shedding and associated factors in COVID-19 patients[25,26].

The primary clinical outcome assessed in this study was LOC. COVID-19 patients with vitamin D deficiency and insufficiency showed a significant effect on shortened hospital stay (11.63±2.5 vs. 14.73±3.45; P = 0.001). Research conducted by Liu et al in Wuhan, China, the average LOS is 11 days[12],

while the study in Germany by Ludwig et al, LOS for COVID-19 patients is about 8 days[27]. A cohort study conducted by Moriconi et al (2020) found that obese COVID-19 patients exhibited a longer LOS compared to non-obese COVID-19 patients, while obese patients had an increased prevalence of respiratory diseases[28].

Clinical manifestations in the 10,000 UI group showed improvement, especially shortness of breath (P = 0.011) and nausea (P = 0.045). The use of O2 support was dominated by nasal cannula and there was improvement (without oxygen modality (P = 0.009). As for oxygen saturation, other respiratory, gastrointestinal, musculoskeletal, and neurological symptoms were also found to improve, but not significant, where the conversion was negative but the patient still felt the residual symptoms. The clinical manifestations of individuals diagnosed with COVID-19 can predominantly be characterized by a cluster of flu-like symptoms (fever, cough, dyspnea, myalgia, fatigue, diarrhea, and olfactory/taste disturbances), but asymptomatic cases have also been cured[4]. Symptoms also usually begin with a non-specific syndrome including fever, dry cough, and fatigue. Several organ systems may be involved, including respiratory (cough, shortness of breath, sore throat, rhinorrhea, hemoptysis, and chest pain), gastrointestinal (diarrhoea, nausea, and vomiting), musculoskeletal (myalgia), and neurological (headache or confusion)[11,4,11,25,29]. The more common signs and symptoms were fever (83–98%), cough (76–82%) and shortness of breath (31–55%). After the onset of illness, the median time to first hospital admission was 7 days. Patients with severe disease, progressing to ARDS and deteriorating in a short time, die from multi-organ failure. The mortality rate of hospitalized patients at the onset of the disease is 11-15%, but then decreases by about 2-3%[12,21,22].

To assess the relationship between the intervention of vitamin D supplementation on functional capacity using HGS, significant results were found in both post-intervention groups with HGS values (6.61±3.01 vs. 4.04±4.44; P = 0.011). The ability to grip is one of the most important functions of the hand, and even grip strength can be used to reflect overall muscle strength. Several studies have shown that grip strength is associated with functional impairment, frailty, impaired cognitive function, physical disability, and nutritional status. In a study among men over 50 years of age, the observed HGS per body weight associated with serum 25(OH)D concentrations was 0.523 (0.430-0.638), 0.545 (0.447-0.664), 0.543 (0.446-0.661), 0.546 (0.449-0.664) (P<0.01)[15].

In the case of COVID-19, a decrease in peripheral TLC has been widely observed and is associated with a severe clinical course. In this study, there was an increase in pre and post intervention TLC levels (P=0.004). TLC reached their lowest values when levels of inflammatory cytokines were highest on days 4-6. Vitamin D deficiency may have a role in hyperinflammation. There is an association between COVID-19 patients and the presence of lymphopenia (37.7%). This is because most viruses when infecting humans cause lymphocyte depletion which can be either direct viral attack on lymphocytes or by immune-mediated lymphocyte apoptosis. In addition, infection by COVID-19 induces the production of multiple cytokines such as IL-7, IL-2, IL-6, TNF-α, and IFN-ɤ known as cytokine storm and can cause lymphocyte apoptosis in addition to lymphoid organ atrophy[3,5,30].

In this study, there was a significant increase in NLR (P<0.0001). Meanwhile, for the analysis of changes in NLR levels were not significant, as well as the analysis of the relationship between NLR and LOC and LOS (P=0.894). The reduction in NLR was confirmed by a placebo-controlled double-blind RCT with an intervention of oral vitamin D3 supplementation (3000-6000 IU/day for 30 days). Increased NLR correlated significantly with LOS in intensive care and mortality[5].

An increase in PLR showed a tendency to be associated with disease progression (hazard ratio [HR] 1.023, 95% CI 0.921–1.756 by multivariate Cox regression), but statistical significance was lost after adjustment for sex and age, limiting its clinical utility[31]. Corona viruses can cause thrombocytopenia by direct viral infection in bone marrow hematopoietic stem cells via CD13 or CD66a, formation of auto-antibodies and immune complexes, disseminated intravascular coagulopathy (DIC), and consumption of platelets in the lung epithelium. Dissolved vascular cell adhesion molecule-1 (sVCAM-1) was found to be higher in SARS patients, which increased vascular sequestration leading to thrombocytopenia[5,6,31,32].

Analyzes were performed on both groups of changes in D-Dimer levels, the use of anticoagulants, and coagulopathy. D-dimer levels increase in the acute phase of COVID-19 infection, and are used today to determine the severity of ongoing disease. The thrombus is composed of fibrin together with platelets, GP Ib, GP IIb/IIIa, von Willebrand factor and tissue factor (collagen). The presence of a thrombus that clogs blood flow makes the body perform homeostasis to destroy the thrombus. D-dimer is the end product of fibrin breakdown by plasmin. Another study reported that administration of vitamin D was associated with levels of D-Dimer and Immune Reconstitution Inflammatory Syndrome (IRIS)[6,7,33].

The strength of this clinical trials conducted involving moderate COVID-19 patients focused on low vitamin D status, while the limitation that was not measure calcium levels pre-post intervention, but no side effects were reported during the intervention period. It is necessary to monitor in a regular basis for whom was supplemented by therapeutic doses of vitamin D to achieve sufficiency status in COVID-19 patients. Further research is expected to be carried out in a period of more than 2 weeks or with larger doses of vitamin D3 to assess the efficacy and effectiveness, especially in patients with severe or critical conditions, complications, and post-COVID syndrome.

CONCLUSION

The effects of 10,000 IU Vitamin D3 supplementation in moderate COVID-19 patients whom have low vitamin D status showed improvements in LOC, LOS, clinical manifestations, functional capacity, and positive correlation on inflammatory and coagulation markers, such as PLR.

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