Int J Nutr Sci 2024;9(4):260-268

International Journal of Nutrition Sciences

Journal Home Page: ijns.sums.ac.ir

ORIGINAL ARTICLE

The Effect of High-Dose Vitamin D on Inflammatory Markers of ICU Patients with COVID-19

Mohammad Hossein Zamanian[1], Zohreh Javadfar[2,3*], Shima Moradi[3,4], Roghayeh Mostafaei[3,4], Ali Soroush[5], Sahar Cheshmeh[6], Akram Rahimi[2*]

1. Department of Infectious Diseases, School of Medicine, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
3. Department of Nutrition Sciences, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran
5. Department of Sports Medicine, Cardiovascular Research Center, Imam Ali Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
6. Department of Molecular and Experimental Nutritional Medicine, University of Potsdam, Nuthetal, Germany

ARTICLE INFO

Keywords:
Vitamin D
C-reactive protein
Interleukin-6
COVID-19
Iran

**Corresponding author:*
Akram Rahimi, MSc;
Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Tel: +98-9184801501
Email: akramrahimi366@gmail.com
Zohreh Javadfar, MSc;
Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Tel: +98-9181317141
Email: zohre.javadfar@yahoo.com
Received: July 10, 2024
Revised: October 4, 2024
Accepted: October 14, 2024

ABSTRACT

Background: Vitamin D can inhibit hyper-inflammatory responses, and there are mechanistic reasons for vitamin D's beneficial effects in COVID-19 patients. The aim of the present study was to assess how a single high dose of vitamin D can affect inflammatory markers among COVID-19 patients in critical conditions.

Methods: A single center, double-blind, randomized clinical trial was conducted on 61 COVID-19 patients admitted to the intensive care units (ICUs). The intervention group received 300,000 IU vitamin D intramuscularly and identically placebo patients were monitored for one week. The levels of lactate dehydrogenase (LDH, IU/mL), C-reactive protein (CRP, mg/L), interleukin-6 (IL-6, Pg/L), lymphocytes, neutrophils, and neutrophil/lymphocyte (N/L) ratios were measured at the start and end of the study.

Results: This trial included 44 patients with COVID-19 who had vitamin D deficiency at the start. After a high vitamin D dose intervention, lymphocyte level increased while LDH (IU/mL) and neutrophil levels, and N/L ratio decreased. CRP (mg/L) and IL-6 (Pg/L) levels significantly declined following intervention with a high-dose vitamin D.

Conclusion: These findings suggest that high doses of vitamin D may improve inflammatory indices in COVID-19 ICU patients.

Please cite this article as: Zamanian MH, Javadfar Z, Moradi S, Mostafaei R, Soroush A, Cheshmeh S, Rahimi A. The Effect of High-Dose Vitamin D on Inflammatory Markers of ICU Patients with COVID-19. Int J Nutr Sci. 2024;9(4):260-268. doi: 10.30476/ijns.2024.102550.1320.

Introduction

COVID-19 is caused by a new coronavirus known as severe acute respiratory syndrome coronavirus (SARS-CoV-2), which emerged in China at the end of 2019 and quickly spread throughout the world. COVID-19 still remains a significant threat to human health in the majority of countries (1). The adverse effects of COVID-19 were attributed to immune dysregulation, which resulted in an increase in pro-inflammatory mediators (a cytokine storm)

(2). As COVID-19 progresses, pro-inflammatory cytokines are released, causing lung inflammation and fibrosis. Immune system modulators, on the other hand, can lessen the severity and improve outcomes (2). Many studies have shown that complementary medicine and natural compounds, as well as nutritional supplements are effective in controlling inflammation and oxidative stress, and thus improving clinical outcomes in critically ill patients (3-5). Most studies have found a link between an increased risk of respiratory diseases, including COVID-19 and lack of vitamin D (6-8). Vitamin D's beneficial effects in COVID-19 have been attributed to immunomodulatory and anti-inflammatory properties, particularly in viral infections (9). Vitamin D has been shown to improve both innate and adaptive immune function receptors in the respiratory tract, as well as to protect against inflammation by suppressing tumor necrosis factor-α (TNF-α) and inflammatory cytokines in the severe form of COVID-19 and by increasing anti-inflammatory cytokines (9-11). In lung epithelial cells, the active form of vitamin D increases gene expression of cathelicidin, which is an antimicrobial protein in the body. Cathelicidin inhibits virus survival and multiplication as well as the synthesis of pro-inflammatory cytokines, thereby protecting lung tissue from inflammation (11).

Vitamin D deficiency has been reported among 45.6% of Iranian men and 61.9% of Iranian women (12). So vitamin D supplementation may be a useful strategy for preventing and treating COVID-19 or alleviate its symptoms (4). There are lacks of effective pharmaceutical interventions in preventing and treating the disease and to improve immune responses to eliminate the virus in its early stages. Changes in diet, lifestyle by intake of vitamin D were shown to improve the immune system, alter the severity of COVID-19, and increase the likelihood of contracting COVID-19 (4, 13). Therefore, the purpose of this study was to look for relationship between vitamin D supplementation and inflammatory markers in COVID-19 patients admitted to the intensive care units (ICUs) of Golestan Hospital in Kermanshah, Iran.

Materials and Methods

Based on the CONSORT statement (14), this single center, randomized, double-blind, placebo-controlled clinical trial was designed to assess the effect of high-dose vitamin D on inflammatory status among COVID-19 patients admitted to the ICU ward of Golestan Hospital in Kermanshah, Iran. Based on Mirolyaei *et al.*'s study (15), a sample size of 15 patients was determined (Confidence interval: 95%, test power: 90%). However, due to the nature of the disease and the possibility of high drop-out, the sample size was limited to 30 patients. All procedures involving human participants were carried out in accordance with the institutional and/or national research committee's ethical standards, as well as the 1964 Helsinki declaration and its subsequent amendments or comparable ethical standards. The Ethics Committee of Kermanshah University of Medical Sciences approved the study (Ethics approval number: IR.KUMS.MED.REC.1400.069; Iranian Clinical Trials Registry number: IRCT20170827035936N2. The trial registration: IRCT20170827035936N (19/12/2021). After explaining the purpose of the study, each subject provided a written informed consent. Subjects had the right to withdraw from the study at any time, and their information was confidential and promised not to be published.

COVID-19 patients older than 18 years old diagnosed with flu symptoms via computed tomography scan (CT scan) or by nasopharyngeal swabs in real-time polymerase chain reaction (RT-PCR) and hospitalized patients in ICU were excluded from the study. Intake of 25 (OH) vitamin D at levels greater than 30 ng/dL, a previous vitamin D (1000 IU/d) supplementation, unwillingness to continue cooperation in the study, already receiving invasive mechanical ventilation or in-hospital mortality within the first week of intervention, pregnancy, lactation, renal failure (creatinine≥2 mg/dL) and hypercalcemia (calcium≥10 mg/dL) were considered as exclusion factors. Patients were randomly assigned to either the vitamin or placebo groups in a 1:1 ratio. A combination of four and six computer-generated random blocks were randomly used to divide patients to intervention (n=31) and placebo (n=30) groups (Figure 1). The intervention group received a single intramuscular injection of 300,000 IU along with standard treatment. The placebo group received the same dose of an intramuscular injection placebo by Daroupakhsh Pharmaceutical Company, Tehran, Iran, with the exception of vitamin D as the main ingredient and standard treatment. Patients were monitored for one week until the final analysis, while both patients and investigators were unaware of the randomization. To assess matching in the intervention and placebo groups, demographic information, clinical symptoms of disease, vital signs including temperature, pulse rate (PR), respiratory rate (RR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and medication use during hospitalization were collected. The hospital information system was used to provide the data on individual-level covariates, such as age, gender,

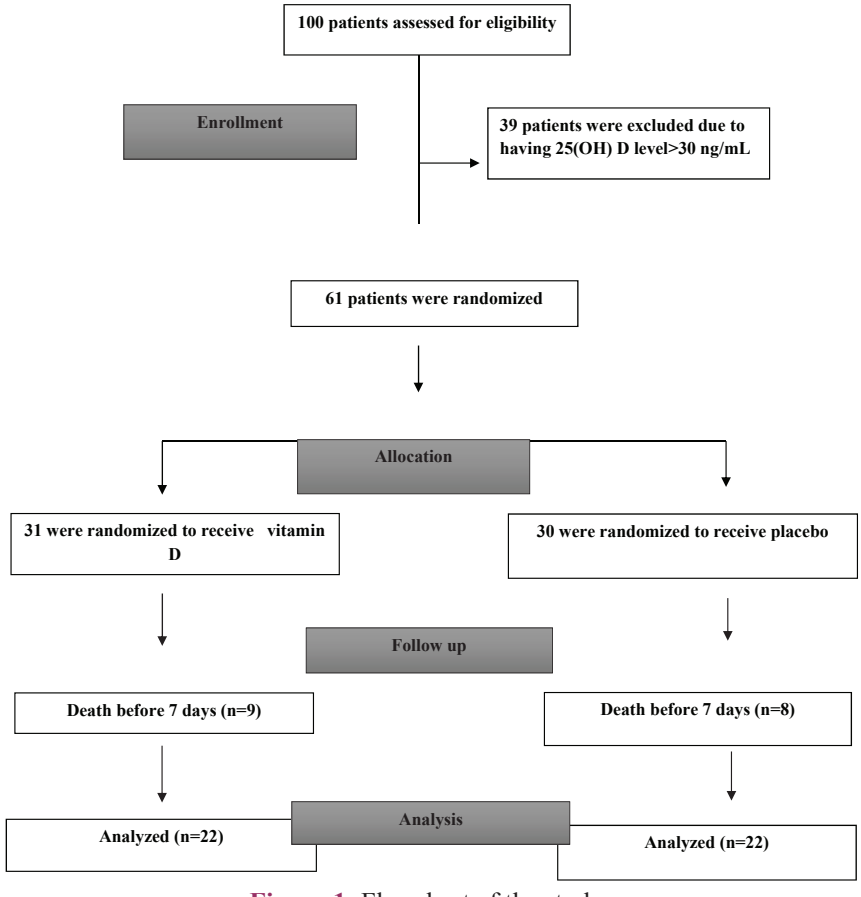

Figure 1: Flowchart of the study.

weight, height, body mass index (BMI), acute covid19 symptoms, duration of symptoms, and medications. All stages of the study were carried out by trained experts in terms of the method of conducting the study.

Lactate dehydrogenase (LDH), albumin, C-reactive protein (CRP), Interleukin–6 (IL-6), lymphocytes, neutrophils, and 25(OH)D were measured at the start and end of the study (on the seventh day). Mindray BC-6800 was used to count lymphocytes and neutrophils. As an inflammatory index, the lymphocyte/neutrophil (N/L) ratio was calculated too. LDH was determined utilizing the Mindray BS-800M machine (Farasamed Persian Laboratory Kit-Iran). Albumin was assessed employing the Mindray BS-800M machine (Farasamed Persian Laboratory Kit-Iran), and CRP was evaluated applying the immuno-turbidimetry technique (Auto analyzer-Cs/1200). The Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the levels of vitamin D and IL-6 in the serum samples.

The SPSS software (Version 19, Chicago, IL, USA) was used to analyze the data and the Kolmogorov-Smirnov test determined the data's normality. The characteristics of the patients were reported as descriptive statistics. The Chi-square test was utilized to assess the difference between the two groups' qualitative variables. Mann-Whitney and independent sample T-Tests compared the two groups' anthropometric and biochemical indices. The difference between the variables between the studied groups was evaluated by employing the paired samples T-test or the Wilcoxon test. Covariance (ANCOVA) analysis was applied to adjust confounding variables (baseline variable of CRP). A p-value of less than 0.05 was regarded significant.

Results

The mean age of the intervention and control groups was 56.33±14.65 and 50.65±11.4 years, respectively, with no statistically significant difference between the two studied groups (p=0.158). Men included 77.3% and 63.6% of the intervention and control groups, respectively; while the difference was not statistically significant between the two groups (p=0.255). The mean BMI of the intervention and placebo groups was 28.72±5.18 and 30.88±4.88 kg/m^2, respectively, with no significant difference (p=0.078). There was no statistically significant difference in serum albumin level between the intervention and placebo groups (p=0.642). No significant difference was found for medications prescribed for the patients in the two groups (p=0.5). The vital signs of temperature, RR, PR,

SBP, and DBP did not differ significantly between the two groups. Table 1 summarizes the basic characteristics of the COVID-19 patients. There was no significant difference for clinical symptoms associated with COVID-19 between the two groups; while all patients complained of sore throat (Table 2). The COVID-19 endangered the lives of nine patients in the intervention group and eight in the placebo group. As a result, this trial was completed with 44 COVID-19 patients (22 in intervention and 22 in placebo, Figure 1).

The serum 25 (OH) D3 (ng/mL) level was significantly higher in the intervention group (p<0.001, Figure 2). Furthermore, the lymphocyte count increased (p=0.817); while the neutrophil level (p=0.234) and N/L ratio (p=0.465) decreased (Figure 3a). The opposite occurred in the placebo group, but none of these differences were statistically significant (Table 3). The changes in LDH (IU/mL) level were not significant in either group (Table 3, Figure 3b). CRP (mg/L) was significantly low in both groups, but the reduction in intervention group was significantly greater than the placebo group (p=0.035, Table 3 and Figure 3c). Furthermore, after intervention with a high dose of vitamin D, IL-6 (Pg/L) significantly decreased (p=0.031, Table 3 and Figure 3d).

Table 1: Basic characteristics of patients with COVID-19.

Variable	Intervention (n=22)	Placebo (n=22)	*P* value*
Age (year)	56.33±14.65	50.65±11.40*	0.158
Gender, male (%)	77.3	63.6	0.255
Duration of symptom (days)	8.33±2.58	8.32±2.75	0.985
Weight (kg)	81.27±16.64	89.59±16.33	0.102
BMI (kg/m^2)	28.72±5.18	30.88±4.88	0.078
T (°C)	36.80±0.43	36.70±0.56	0.697
PR	97.27±15.35	94.68±15.82	0.584
RR	20.00±3.56	20.71±2.41	0.191
SBP (mmHg)	119.36±15.34	117.22±14.41	0.636
DBP (mmHg)	74.91±9.70	73.04±9.06	0.514
Alb (IU/L)	3.41±0.26	3.44±0.32	0.642
Medication (%)			
Remdesivir	100	100	.
Anticoagulants (Heparin or enoxaparin)	100	100	.
Antibiotics (Ranging from imipenem to ciprofloxacin)	100	100	.
Methylprednisolone Q2 saturation<90%	95.5	90.9	0.5
Dexamethasone Q2 saturation>90%	4.5	9.1	0.5

BMI: Body mass index; T: Temperature; PR: Plse rate; RR: Respiratory rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; Alb: Albumin. *Mean±SD. P* was obtained from Chi-square, Mann-Whitney U, and independent sample T-Test.

Table 2: Clinical symptoms of patients with COVID-19.

Variable	Intervention (n=22) No. (%)	Placebo (n=22) No. (%)	*P* value*
Weakness and lethargy	11 (52.4)	7 (31.8)	0.145
Fever	2 (9.5)	0 (0)	0.233
Shivering	13 (61.9)	17 (77.3)	0.223
Cough	14 (66.7)	10 (45.5)	0.137
Dyspnea	15 (71.4)	14 (63.6)	0.414
Headache	3 (14.3)	5 (22.7)	0.376
Dizziness	1 (4.8)	2 (9.1)	0.518
Hemoptysis	3 (14.3)	0 (0)	0.108
Diarrhea	0 (0)	1 (4.5)	0.512
Vomit	0 (0)	3 (13.6)	0.125
Nausea	1 (4.8)	3 (13)	0.321
Stomachache	0 (0)	1 (4.3)	0.512
Anorexia	2 (9.5)	2 (8.7)	0.679
Sore throat	21 (100)	22 (100)	.

Mean±SD. P was obtained from Chi-square.

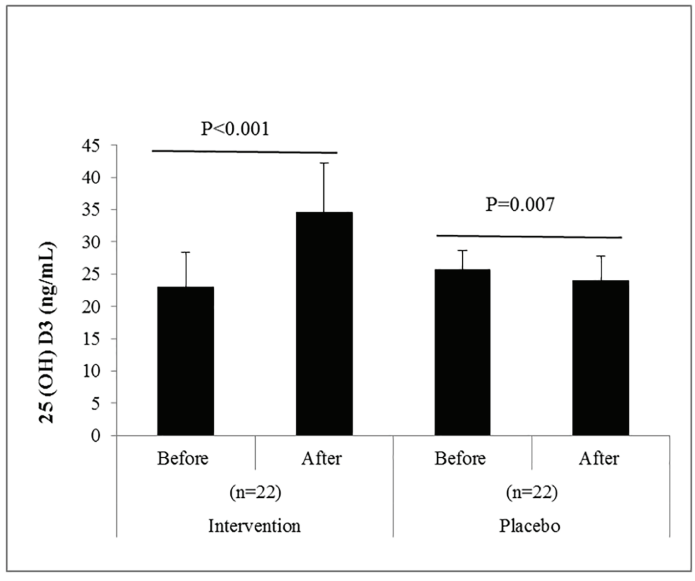

Figure 2: Changes of 25 (OH) D3 (ng/mL) before and after interventions with a high dose of vitamin D.

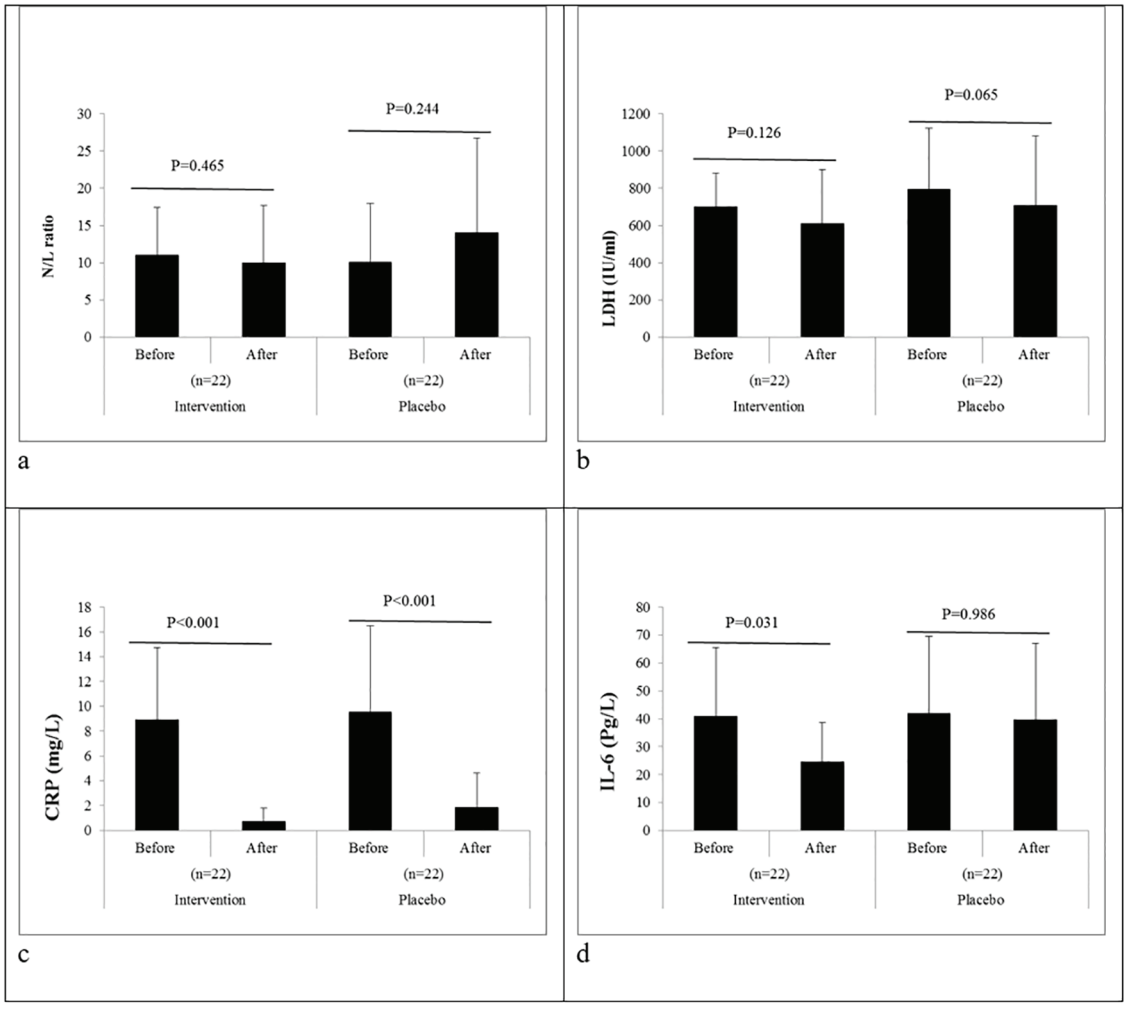

Figure 3: Changes of a) N/L ratio; b) LDH (IU/mL); c) CRP (mg/L); and d) IL-6 (Pg/L) before and after interventions with a high dose of vitamin D.

Discussion

A single high dose of vitamin D was demonstrated to have a positive effect on inflammatory markers in ICU patients with COVID-19 who were already vitamin D deficient. The COVID-19 can have a negative impact on respiratory system and cause a flood of pro-inflammatory cytokines (16). In these patients, suppression of the inflammatory condition is a critical therapeutic strategy (17). To advance our understanding, we investigated the effect of

Table 3: Biochemical changes after intervention with a high dose of vitamin D in patients with COVID-19.

Variable	Intervention (n=22) (Mean±SD)		P1	Placebo (n=22) (Mean±SD)		P1	P2	P3
	Before	After		Before	After			
Lymphocyte (%)	10.87±6.56	11.27±4.90	0.817	11.28±6.08	9.45±4.67	0.279	0.833	0.213
Neutrophil (%)	85.19±8.13	82.65±6.46	0.234	84.31±6.46	85.13±6.19	0.698	0.694	0.201
N/L ratio	10.97±6.46	9.93±7.74	0.465	10.01±7.94	14.05±12.71	0.244	0.432	0.280
LDH (IU/mL)	701.14±179.78	608.24±290.40	0.126	794.14±326.90	708.59±371.16	0.065	0.536	0.528
25 (OH) D3 (ng/mL)	23.06±5.33	34.53±7.66	<0.001	25.68±3.01	24.00±3.81	0.007	0.051	<0.001
CRP (mg/L)	8.93±5.79	0.72±1.06	<0.001	9.52±6.94	1.87±2.75	<0.001	0.020	0.035
IL-6 (Pg/L)	40.79±24.79	24.58±14.11	0.031	41.99±27.63	39.56±27.37	0.986	0.842	0.076

N/L ratio: Neutrophil/ Lymphocyte ratio; LDH: Lactate dehydrogenase; CRP: C-reactive protein; IL-6: Interleukin 6; *All presented values are means±SD. P1; P values denote the significance of within-group changes. P2: P values denote the significance of between-group differences before intervention. P3: P values denote the significance of between-group differences after the intervention. *Significant difference within the group throughout the study (p<0.05, paired samples t-test or Wilcoxon). *Significant difference between groups throughout the study (p<0.05, independent samples t-test or U Mann Whitney).

high-dose vitamin D on inflammatory factors in COVID-19ICU patients. Vitamin D is a fat-soluble vitamin with extra-musculoskeletal effects, such as anti-fibrotic, anti-inflammatory, and antioxidant properties (4, 18). Adequate intake of this vitamin can prevent the expression of pro-inflammatory cytokines, whereas its deficiency results in production of inflammatory cytokines (18).

After supplementation with a high-dose vitamin D, the lymphocyte count increased; while the number of neutrophils and N/L ratio decreased. Lakkireddy *et al.* found that taking 60,000 IUs of vitamin D per day for 8-10 days could significantly reduce N/L ratio (p<0.05) (19). Another study conducted by Maghbooli *et al.* revealed that taking 3000 to 6000 IU of vitamin D3 per day significantly declined the N/L ratio in COVID-19 patients (20). The N/L ratio is a readily available and inexpensive biomarker that reflects the patient's inflammatory status. Furthermore, the N/L ratio is a biomarker for a variety of diseases, including tumors, pancreatitis, diabetes, chronic obstructive pulmonary disease (COPD), and cardiovascular diseases (21-23). Some studies have illustrated that this biomarker can help classify the severity of the disease and mortality caused by COVID-19 (24-26). Lymphopenia is seen in the majority of viral infections, including COVID-19, and is related to the disease severity (6). In these patients, vitamin D suppresses pro-inflammatory responses by improving the interaction of immune system cells such as neutrophils, monocytes, mast cells, and macrophages and decreasing the neutrophil/lymphocyte ratio (27-29).

Other inflammatory indices, such as LDH, CRP, and IL-6 were low in both groups. The reduction in inflammatory indices happened because both groups received anti-inflammatory drugs. The decrease in CRP level after the intervention with a high dose of vitamin D, on the other hand, was significantly greater than the placebo group. Furthermore, the reduction in IL-6 in the intervention group was significant, whereas the reduction in the placebo group was small and not significant. Vitamin D deficiency has been shown to be linked to an increase in inflammatory cytokines such as CRP and IL-6 (30, 31). Adequate vitamin D levels can boost the immune system and promote innate immunity against viral infections by secreting specific peptides (32, 33). Vitamin D deficiency is associated with acute infections in respiratory system and epidemics such as influenza (34, 35). Vitamin D acts as a physical barrier against viral infections, and promotes innate and adaptive immunity (36). Furthermore, vitamin D protects cell connections and lowers levels of pro-inflammatory cytokines (37). Vitamin D improves adaptive immunity in viral infections of respiratory system by increasing the levels of virus-specific CD8+ T cells, C-X-C Motif Chemokine 10, and interferon in the respiratory epithelium, through recruiting immune cells to the infection site and via decreasing viral helix replication (38).

Our study was a well-designed double-blind, randomized clinical trial in which we controlled for many confounding factors, such as patient's vital signs, medications, and disease symptoms, and randomly allocated patients to two groups. Furthermore, we only looked at patients admitted to the ICU with critical COVID-19 and vitamin D deficiency. However, several issues necessitate additional researches to address these limitations such as the study sample size that was small, with only one center in Kermanshah, Iran. Furthermore, patients with COVID-19 frequently died in the ICU, and accessing patients with similar conditions was difficult. In fact, the main limitation

was missing the clinical care of included patients. Beside the positive role of vitamin D supplements in critical patients with Covid-19, there was a high dependency on receiving relevant treatments including antibiotics, corticosteroids, and anti-inflammatories in case of clinical indication. To overcome this problem, we matched both groups in terms of receiving relevant treatments including antibiotics, corticosteroids, anti-inflammatories, clinical symptoms and even some biochemical parameters as confounder factors. Another critical issue was that all of these patients were initially vitamin D deficient, and the final point was that the intervention was brief in course and as a result, significant changes in some factors could not be observed. Therefore, all of these limitations can make it difficult to generalize the findings to all COVID-19 patients.

Conclusion

According to the findings, high doses of vitamin D improved the inflammatory condition and reduced CRP and IL-6 in COVID-19 ICU patients. Furthermore, the lymphocyte count increased, while the neutrophil count and N/L ratio decreased. However, when compared to the control group, the results favored COVID-19, which was insignificant. As a result, this study supported the hypothesis regarding vitamin D's anti-inflammatory effects in the COVID-19 crisis.

Acknowledgment

The authors would like to thank all patients who participated in this study for their valuable contributions. We would like to thank the Kermanshah University of Medical Science for financial support (Grant No: 4000823).

Authors' Contribution

AR and ZJ contributed in conception and design of the research; AR, MZ, AS, and ZJ contributed to data collection; SM and RM contributed to the acquisition and analysis of the data; SM and ZJ contributed to the interpretation of the data; SM, RM, and ZJ contributed to writing the draft of the manuscript. SM, RM, ZJ, and SC contributed to critically revise of the manuscript. All authors are in agreement with the manuscript and declare that the content has not been published elsewhere. The authors of AR and ZJ contributed equally.

Conflict of Interest

None declared.

References

1 Almasi F, Nemati M, Rabiee R, et al. The Correlation between Nuts Consumption and Severity and Symptoms of COVID-19. *Int J Nutr Sci*. 2024;9:236-244. DOI: 10.30476/ijns.2024.102820.1327.

2 Ghadimi Moghadam AK, Masoumi SJ, Nouri M, et al. The Effect of Nutrients Intake with an Emphasis on Immune-Boostings in Patients with COVID-19. *Int J Nutr Sci*. 2022;7:34-40. DOI: 10.30476/IJNS.2022.94174.1170.

3 Mehrdad M, Eftekhari MH. Concerns on Obesity during COVID-19 Pandemic. *Int J Nutr Sci*. 2021;6:111-112. DOI: 10.30476/IJNS.2021.90311.1125.

4 Rooiqoh QF, Tamtomo DG, Cilmiaty R. The Relationship of Carbohydrate, Vitamin D, Zinc Consumption and Physical Activity with Fasting Blood Glucose Level in Type 2 Diabetes Mellitus Patients during COVID-19 Pandemic. *Int J Nutr Sci*. 2023;8:20-26. DOI: 10.30476/IJNS.2023.97025.1206.

5 Ahmadi S, Firoozi D, Masoumi SJ. The Effect of Micronutrients on COVID-19 Disease: A Review of Available Evidences. *Int J Nutr Sci*. 2022;7:10-18. DOI: 10.30476/IJNS.2022.94162.1169.

6 Mardani R, Alamdary A, Mousavi Nasab SD, et al. Association of vitamin D with the modulation of the disease severity in COVID-19. *Virus Res*. 2020;289:198148. DOI: 10.1016/j.virusres.2020.198148. PMID: 32866536.

7 Siddiqui M, Manansala JS, Abdulrahman HA, Nasrallah GK, Smatti MK, Younes N, et al. Immune Modulatory Effects of Vitamin D on Viral Infections. *Nutrients*. 2020;12.

8 Afaghi S, Esmaeili Tarki F, Sadat Rahimi F, Besharat S, Mirhaidari S, Karimi A, et al. Prevalence and Clinical Outcomes of Vitamin D Deficiency in COVID-19 Hospitalized Patients: A Retrospective Single-Center Analysis. *Tohoku J Experiment Med*. 2021;255:127-34.

9 Mirzay Razaz J, Nosrati-Oskouie M, Hassan Qomi M, et al. Practical Recommendations of Micronutrients and Food Hygiene for Rehabilitation of Survived COVID-19 Patients: A Review. *Int J Nutr Sci*. 2021;6:59-64. DOI: 10.30476/IJNS.2021.88587.1099.

10 Mirzay Razaz J, Nosrati-Oskouie M, Hassan Qomi M, et al. Nutritional Support for Rehabilitation of Survived COVID-19 Patients: A Review. *Int J Nutr Sci*. 2021;6:1-5. DOI: 10.30476/IJNS.2021.87600.1085.

11 Aygun H. Vitamin D can prevent COVID-19 infection-induced multiple organ damage. *Naunyn Schmiedebergs Arch Pharmacol*. 2020;393:1157-60. DOI: 10.1007/s00210-020-01911-4. PMID: 32451597.

12 Tabrizi R, Moosazadeh M, Akbari M, et al. High prevalence of vitamin D deficiency among Iranian population: a systematic review and meta-analysis. *Iran J Med Sci.* 2018;43:125.
13 Yücel ÜÖ, Kömbeci K, Tuğrul G, et al. Changes in Diet, Lifestyle and Orthorexia Nervosa Attitudes of Vegetarian, Vegan and Omnivorous Individuals in COVID-19 Pandemic. *Int J Nutr Sci.* 2021;6:74-80. DOI: 10.30476/IJNS.2021.90358.1126.
14 Boutron I, Moher D, Altman DG, Schulz KF, et al. Extending the CONSORT statement to randomized trials of nonpharmacologic treatment: explanation and elaboration. *Ann Intern Med.* 2008;148:295-309.
15 Miroliaee AE, Salamzadeh J, Shokouhi S, Sahraei Z. The study of vitamin D administration effect on CRP and Interleukin-6 as prognostic biomarkers of ventilator associated pneumonia. *J Critical Care.* 2018;44:300-5. DOI: 10.1016/j.jcrc.2017.08.040.
16 Conti P, Ronconi G, Caraffa A, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents.* 2020;34:327-31.
17 Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol.* 2020;20:363-74. DOI: 10.1038/s41577-020-0311-8. PMID: 32346093.
18 Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin D supplementation: Evidence from human studies. *Nat Rev Endocrinol.* 2022;18:96-110. DOI: 10.1038/s41574-021-00593-z. PMID: 34815552.
19 Lakkireddy M, Gadiga SG, Malathi R, et al. Impact of daily high dose oral vitamin D therapy on the inflammatory markers in patients with COVID 19 disease. *Scientific Rep.* 2022;12. DOI: 10.1038/s41598-021-90189-4.
20 Maghbooli Z, Sahraian MA, Jamalimoghadamsiahkali S, et al. Treatment With 25-Hydroxyvitamin D3 (Calcifediol) Is Associated With a Reduction in the Blood Neutrophil-to-Lymphocyte Ratio Marker of Disease Severity in Hospitalized Patients With COVID-19: A Pilot Multicenter, Randomized, Placebo-Controlled, Double-Blinded Clinical Trial. *Endocrine Practice.* 2021;27:1242-51. DOI: 10.1016/j.eprac.2021.09.016.
21 Russell CD, Parajuli A, Gale HJ, et al. The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: A systematic review and meta-analysis. *J Infection.* 2019;78:339-48. DOI: 10.1016/j.jinf.2019.02.006.
22 Parthasarathi A, Padukudru S, Arunachal S, et al. The Role of Neutrophil-to-Lymphocyte Ratio in Risk Stratification and Prognostication of COVID-19: A Systematic Review and Meta-Analysis. *Vaccines.* 2022;10:1233. DOI: 10.3390/vaccines10081233.
23 Aghasadeghi K, Zarei-Nezhad M, Keshavarzi A, et al. The prevalence of coronary risk factors in Iranian lor migrating tribe. *Arch Iran Med.* 2008;11:322-5. PMID: 18426325.
24 Zinellu A, Mangoni AA. A systematic review and meta-analysis of the association between the neutrophil, lymphocyte, and platelet count, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio and COVID-19 progression and mortality. *Expert Rev Clin Immunol.* 2022;18:1187-202. DOI: 10.1080/1744666X.2022.2120472. PMID: 36047369.
25 Simadibrata DM, Calvin J, Wijaya AD, et al. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: A meta-analysis. *Am J Emerg Med.* 2021;42:60-9. DOI: 10.1016/j.ajem.2021.01.006. PMID: 33453617.
26 Wang Y, Zhao J, Yang L, et al. Value of the Neutrophil-Lymphocyte Ratio in Predicting COVID-19 Severity: A Meta-analysis. *Dis Markers*. 2021;2021:2571912. DOI: 10.1155/2021/2571912. PMID: 34650648.
27 Teymoori□Rad M, Marashi SM. Vitamin D and Covid□19: from potential therapeutic effects to unanswered questions. *Rev Med Virol.* 2021;31:e2159. DOI: 10.1002/rmv.2159. PMID: 32856339.
28 Tabatabaeizadeh SA, Avan A, Bahrami A, et al. High dose supplementation of vitamin D affects measures of systemic inflammation: Reductions in high sensitivity C□reactive protein level and neutrophil to lymphocyte ratio (NLR) distribution. *J Cell Biochem*. 2017;118:4317-22. DOI: 10.1002/jcb.26084. PMID: 28425575.
29 Yip KH, Kolesnikoff N, Yu C, et al. Vitamin D3 represses IgE-dependent mast cell activation via mast cell-CYP27B1 and-vitamin D receptor activity. *J Allergy Clin Immunol.* 2014;133:1356-64. DOI: 10.1016/j.jaci.2013.11.030. PMID: 24461581.
30 Manion M, Hullsiek KH, Wilson EM, Rhame F, Kojic E, Gibson D, et al. Vitamin D deficiency is associated with IL-6 levels and monocyte activation in HIV-infected persons. *PLoS One.* 2017;12:e0175517. DOI: 10.1371/journal.pone.0175517. PMID: 28464004.

31 Poudel-Tandukar K, Poudel KC, Jimba M, et al. Serum 25-hydroxyvitamin d levels and C-reactive protein in persons with human immunodeficiency virus infection. *AIDS Res Hum Retroviruses*. 2013;29:528-34. DOI: 10.1089/AID.2012.0120. PMID: 23003113.

32 Wang TT, Dabbas B, Laperriere D, et al. Direct and indirect induction by 1, 25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin β2 innate immune pathway defective in Crohn disease. *J Biol Chem*. 2010;285:2227-31. DOI: 10.1074/jbc.C109.071225. PMID: 19948723.

33 Ali N. Role of vitamin D in preventing of COVID-19 infection, progression and severity. *J Infect Public Health*. 2020;13:1373-80. DOI: 10.1016/j.jiph.2020.06.021. PMID: 32605780.

34 Cannell J, Vieth R, Umhau J, et al. Epidemic influenza and vitamin D. *Epidemiol Infec* 2006;134:1129-40. DOI: 10.1017/S0950268806007175. PMID: 16959053.

35 Ginde AA, Mansbach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2009;169:384-90. DOI: 10.1001/archinternmed.2008.560. PMID: 19237723.

36 Rondanelli M, Miccono A, Lamburghini S, et al. Self-care for common colds: the pivotal role of vitamin D, vitamin C, zinc, and echinacea in three main immune interactive clusters (physical barriers, innate and adaptive immunity) involved during an episode of common colds—practical advice on dosages and on the time to take these nutrients/botanicals in order to prevent or treat common colds. *Evid Based Complement Alternat Med*. 2018;2018:5813095. DOI: 10.1155/2018/5813095. PMID: 29853961.

37 Grant WB, Lahore H, McDonnell SL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients*. 2020;12:988. DOI: 10.3390/nu12040988. PMID: 32252338.

38 AlKhafaji D, Al Argan R, Albaker W, et al. The Impact of Vitamin D Level on the Severity and Outcome of Hospitalized Patients with COVID-19 Disease. *Int J Gen Med*. 2022;15:343. DOI: 10.2147/IJGM.S346169. PMID: 35027842.