Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 7 Issue 4 April 2023

Research Article

Mortality and Clinical Outcomes of Hospitalized COVID-19 Patients is Associated with Serum Concentrations of Selenium and Vitamin D

Reihane Taheri[1,6], Hossein Faramarzi[2,3], Maryam Ekramzadeh[1]*, Hadi Raeisi Shahraki[4], Zahra Negarande[5] and Siavash Babajafari[1,5]

[1]Department of Clinical Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

[2]Department of Community Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[3]Health Behavior Science Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[4]Department of Epidemiology and Biostatistics, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran

[5]Nutrition Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[6]Metabolic and Cardiovascular Diseases Laboratory, Division of Human Nutrition, University of Alberta, Edmonton

***Corresponding Author:** Maryam Ekramzadeh, Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.

DOI: 10.31080/ASNH.2023.07.1215

Received: February 16, 2023

Published: March 01, 2023

Abstract

Background: Coronavirus disease (COVID-19) has imposed serious effects on public health. The main defender against this viral inflammatory disease is the body's immune system. Selenium and vitamin D as anti-inflammatory, immune-enhancing micronutrients could be beneficial in alleviating the worst outcomes of COVID-19.

Methods: One hundred hospitalized COVID-19 patients with saturation of oxygen (SpO2) < 94 were assessed. In the first day of admission to the hospital, serum selenium and 25-hydroxy vitamin D concentrations were measured. Other clinical outcomes, including lung involvement, length of hospital stay (LOS), C-reactive protein (CRP), SpO2, intubation, and gastrointestinal and neural symptoms were extracted from each patient's medical record. Twenty-four-hour food recall was taken to evaluate the food intake of patients.

Results: Fifty-six percent of patients were 25-hydroxy vitamin D deficient, and 2 percent were selenium deficient. After adjusting for confounding variables, serum selenium was negatively associated with mortality (coefficient: -0.16, p-value: 0.01) and both selenium (coefficient: -0.10, p-value: 0.01) and 25-hydroxy vitamin D (coefficient: -0.11, p-value: 0.004) showed inverse correlation with LOS.

Conclusion: There is an inverse association between serum concentrations of selenium and 25-hydroxy vitamin D with adverse clinical outcomes and mortality of patients with severe COVID-19. Higher concentrations of selenium were associated with increased SpO2 and decreased LOS and risk of death. Although higher concentrations of 25-hydroxy vitamin D were associated with reduced LOS and percentage of lung involvement, no association was found regarding mortality.

Keywords: Selenium; Vitamin D; COVID-19; Lung Involvement; Mortality

Introduction

COVID-19 has imposed serious effects on both public health and the economy worldwide [1,2]. Although the symptoms are mainly flu-like, this virus can affect many other organs and present various symptoms, including hypogeusia, anosmia, headache, autonomic dysfunction, cardiac involvements, gastrointestinal symptoms, hepatic involvement, acute kidney injury, and dermatologic manifestations [2-14]. Also, the quarantine and isolation lead to panic, anxiety, depression [11,14]. loneliness [4] and obesity [8,15].

Despite efforts to identify a treatment for COVID-19, no definite treatment has been found and preventative measures are still the most effective way [16-19]. In recent months, many vaccines have been approved by the Food and Drug Administration (FDA) for protection against COVID-19; however, new mutations of the virus may lead to ineffectiveness of vaccines [20]. Therefore, attempts to find risk factors for a worse prognosis of the disease and finding a supplementary treatment are still crucial.

The main defender against viral diseases is the body's immune system [21,22]. The immune system relies on many micronutrients for optimal function [21-29], including selenium and vitamin D [24,26-28,30,31]. On the other hand, COVID-19, as a viral infection, triggers a severe immune response and cytokine storm, and leads to systemic inflammation. Thus, the inflammatory response plays a key role in clinical manifestations of COVID-19 [32]. The cytokine and inflammation storms, which occur in some cases, are associated with severe COVID-19, occurrence of acute respiratory distress syndrome (ARDS), and multiple organ failure leading to death in patients with the disease [33-35]. Therefore, decreased level of inflammation in this disease is associated with a lower risk of mortality and severe outcomes, including tissue damage [32]. Selenium and vitamin D are among the micronutrients that have been considered as antioxidant and anti-inflammatory agents [36,37]. Previous studies on the effect of selenium and vitamin D on COVID-19 showed that intravenous selenium supplementation in critically ill COVID-19 patients increased the serum immune cells, including immunoglobulin G, CD8 and natural killer (NK) cells [38]. Vitamin D3 supplementation in geriatric COVID-19 patients increased 3-months survival. In intervention group 76.1% of patients survived at 3 months compared to 53.6% in placebo group (P-value = 0.03) [39]. Pulse vitamin D therapy decreases inflammatory markers [40] and daily oral vitamin D3 therapy decreases the recovery time for cough and ageusia [41] in COVID-19 patients. Their correlation with outcomes of COVID-19 has been partially evaluated in previous studies, but their results are not consistent [42-44]. Especially with regard to selenium level, considering the low sample sizes [45-47], and the retrospective or ecological designs of the studies [45-48]. Moreover, there is no study evaluating the correlation of serum selenium concentration in COVID-19 patients with hospital length of stay, SpO_2, lung involvement, and gastrointestinal and neural symptoms.

We hypothesized that higher concentrations of serum selenium and 25-hydroxy vitamin D is associated with lower occurrence of adverse clinical outcomes and mortality (increased SpO_2, lower risk of mortality, decreased LOS, fever, inflammation (CRP) and lung involvement) in patients with severe COVID-19. Thus, this study was conducted to evaluate the association of serum concentrations of 25-hydroxy vitamin D and selenium with clinical outcomes and risk of mortality in patients with COVID-19.

Materials and Methods

Study Design

This prospective study was conducted on 100 patients with COVID-19 who were admitted to X Hospital (center of care for COVID-19 patients in X, X). Based on the primary outcome of this study (serum selenium concentration), the study of Moghaddam., *et al*. [43], considering the type one error of 0.05, type two error of 0.15, ratio of 1 to 10 (non-survived versus survived), 1.2 as the mean difference between the groups and standard deviations of 1.3 and 0.9, a minimum sample size of 88 (8 dead and 80 alive) was determined. Considering the probability of attrition, 100 eligible patients were collected for the study from February 2021 to March 2021. The inclusion criteria were: X ethnicity, positive test for COVID-19 ribonucleic acid (RNA) with real-time polymerase chain reaction (RT-PCR) within the past five days of admission, first time infection with COVID-19, aged 18-65 years, informed consent of patients participating in the study, $SpO_2 < 94$ (measured by pulse oximeter), and infection with severe COVID-19 requiring hospitalization based on National Institutes of Health (NIH) guidelines [49]. These are defined as $SpO_2 < 94$, or tachypnea (respiratory frequency > 30 breaths/min), or increased alveolar-arterial gradient, or lung involvement > 50% (by computed tomography (CT) scan), or partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) < 300 ($PaO_2/FiO_2 < 300$). The exclusion criteria were smoking, asthma, cancer, diabetes, history of heart attack and stroke, kidney and hepatic diseases, transfer from or to other hospitals (due to disruption of taking blood samples in the first day of admission and possibility of loss of patients for follow-up), lack of consciousness on admission to hospital, and admission to ICU on the first day of hospitalization.

This study was registered at X (Registration Code: in title page) and approved by the research ethics committee of the university (Ethics Approval Code: in title page). All patients participated in the study with written informed consent in accordance with the Declaration of Helsinki.

During the first 24 hours of admission to the hospital, a blood sample was taken of serum 25-hydroxy vitamin D and selenium concentrations and stored for further assessment. The patients were followed until they were discharged or passed away. During this time, any change in patients' status, including admission to ICU, loss of consciousness, need for a ventilator, or other demographic, clinical and dietary data were taken from the patients, their in-charge medical staff, and their medical records.

The blood sample was taken by a qualified nurse. After centrifugation of the blood samples for 15 minutes at the rate of 2500 revolutions per minute (RPM) and relative centrifuge force (RCF) of 2000 g in the hospital's laboratory for serum separation, the ex-

tracted serum was stored in sterile micro-tubes at a temperature of -80°C and sent on dry ice to a remote lab for analysis of selenium and 25-hydroxy vitamin D concentrations. All laboratory technicians were blinded to the outcomes and clinical information. Also, blood samples were taken before the onset of vitamin D therapy in patients.

Serum selenium and vitamin D analysis

Serum selenium concentration was measured by atomic absorption spectrophotometry using a Perkin-Elmer Analyst 300 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) [(HGA 800 (Heated Graphite Atomizer), Autosampler 70 with MHS-10 (Mercury Hydride System)]. For measuring serum 25-hydroxy vitamin D, total 25-hydroxy vitamin D was measured by high-performance liquid chromatography (HPLC). An Agilent 1100 series HPLC system equipped with a ultraviolet (UV) detector was used for the chromatographic analysis (Agilent technologies, Palo Alto, CA, USA). The analytes were separated on a Hector-M C18 4.6 × 150 mm analytical column with 5.0 µm particle size. Quality control (QC) was used for measurement of 25-hydroxy vitamin D and selenium. For QC of selenium assessment, two standard serums (Serum level-1 and level-2, seronorm™) were used; the intra-coefficient of variation (CV) values were 15% and 14.7% and the inter-CV was 5%. For calibration of the atomic gas absorption spectrophotometer, the standard addition calibration method was selected. For QC of 25-hydroxy vitamin D assessment, serum calibrator (25(OH)D, ClinCal®) and controls for 25(OH)D (levels I and II, ClinChek) were used; the inter-CV values were 13% and 10% and the intra-CV value was 0.58%. Selenium and 25-hydroxy vitamin D deficiency were considered as serum selenium concentrations lower than 70 ng/ml [50,51]. and 25-hydroxy vitamin D concentrations lower than12 ng/ml respectively [52]. Insufficiency of vitamin D is defined as serum concentration of 25-hydroxy vitamin D higher than 12ng/ml and lower than 20 ng/ml [52].

Clinical measurements

Demographic, clinical, and outcome data of COVID-19 patients were collected by questionnaires from patients, physical examination findings, and medical records in the hospital. Lung involvement was measured via chest X-rays taken on the first and last days of admission by two blinded radiologists and the final percentage was calculated as the mean of two assessments. During the assessment of lung involvement, the radiologists were blinded to each other's findings and the patients' clinical outcomes. SpO2 was measured using a pulse oximeter on the first and last day of admission (the day of discharge from hospital for those who survived or the day of death for those who did not survive). The data on neural (headache, anosmia, ageusia) and gastrointestinal (nausea, vomiting, diarrhea, constipation, abdominal cramps) symptoms were collected on the first and last days of admission. The neural and gastrointestinal symptoms questionnaire was filled out twice by nurses and was examined by physicians. The first questionnaire was administered by nurses on the first day of admission, asking patients if they had experienced any of the above symptoms during the last five days which lasted more than 12 hours (yes/no question). The second questionnaire, administered on the last day of admission, asked patients if they had experienced any of the above symptoms which lasted more than 12 hours during the hospitalization. Even if a patient complained of only one of the neural/gastrointestinal symptoms, they would be scored yes for having neural/gastrointestinal symptoms. Also, the physicians examined patients' gastrointestinal and neural symptoms and the self-reports of patients were rechecked against the examination. The need for ventilation on admission and during the hospitalization period, respiratory rate (RR), body temperature, admission to Intensive care unit (ICU), loss of consciousness, serum concentrations of magnesium, calcium, potassium, sodium, CRP, Erythrocyte sedimentation rate (ESR), Complete blood count (CBC) with differential, body weight, height, date of admission and discharge, and death were obtained from the patients' medical records. The length of hospital stay was considered from the first day of admission to the day of discharge/death. The exact dosages of patients' medications were not assessed but the principal medications that were used for these patients were dexamethasone and remdesivir, based on their physicians' reports.

Food recall

Three 24-hour food recall questionnaires were filled out by a skilled nutritionist to assess the dietary intake of vitamin D and selenium of the patients during hospitalization to adjust the effect on outcomes of possible different dietary intakes of selenium and vitamin D. Also, the daily dietary sheet of the hospital was checked to ensure the concordance of the patients' self-reports about the type of food that was consumed with the foods delivered by the hospital. The first questionnaire was taken on the first day and then two subsequent questionnaires were taken on alternate days. Lastly, the food recalls were analyzed with NUT4 software. Moreover, all admitted patients without kidney disorders were fed a high-protein diet in the hospital. The patients did not take any selenium supplements, but a daily oral dose of 1000 IU vitamin D was taken by all patients from the first 48 hours of admission to the end of hospitalization.

Statistical analysis

Qualitative and quantitative variables were reported as number (%) and mean ± standard deviation (SD) respectively. To investigate the difference between subgroups, we used independent T-test or ANOVA. To assess the correlation between quantitative variables, we used Pearson correlation. Also, the associations between qualitative variables were examined using Chi square or Fisher's exact test. The association between serum 25-hydroxy vitamin D and selenium concentrations, length of hospital stay, and final outcome (death/discharge) was investigated using linear and logistic regression models respectively. For comparing and determining

the most important variable in linear regression, a standardized coefficient was indicated. All model assumptions were checked and satisfied. Also, for linear models, the residuals were normally distributed and variance inflation factor (VIF) for all the independent variables was less than 5. All statistical analyses were performed using SPSS 21.0 and $P < 0.05$ was considered as statistically significant.

Results

Out of 100 patients, 54 (54%) were male with the mean age of 47.2 ± 11.1 years and with mean body mass index (BMI) of 26.4 ± 4 kg/m^2. The mean length of hospital stay was 7.9 ± 4.8 days, and the final outcome for 10 patients was death (60% male and 40% female). The mean percentage of SpO2 was 87.1 ± 6.9 and that of lung involvement was 28.9 ± 19.9 percent on admission. Also, among all patients, gastrointestinal and neural symptoms were reported in 21 (21%) and 49 (49%) patients respectively. Out of all patients, 13 cases were admitted to ICU and 13 cases needed ventilation. Eight out of 13 patients who were admitted to ICU needed ventilation and were unconscious (Figure 1). Baseline clinical and nutritional characteristics of patients are displayed in table 1.

As shown in table 1, the overall mean of serum selenium and 25-hydroxy vitamin D concentrations were 91.8 ± 11.8 ng/mL and 20.5 ± 12.2 ng/mL respectively. Vitamin D deficiency (lower than 12 ng/mL) was seen in 37% and selenium deficiency (lower than 70 ng/mL) was observed in 2% of the patients. Vitamin D insufficiency (higher than 12 ng/ml but lower than 20 ng/ml) was observed in 20%. For controlling the effect of dietary intake of selenium and vitamin D on the outcomes, we obtained three food recalls and the results of the analyses showed there was no significant difference regarding selenium and vitamin D intake through diet between surviving (113.1 ± 24.1 mg and 2.4 ± 0.5 µg, respectively) and non-surviving patients (117.8 ± 27.8 mg and 2.4 ± 0.4 µg, respectively) ($P = 0.566$, $P = 0.901$). Moreover, the mean intake of energy and protein was not statistically different between the surviving and non-surviving patients ($P = 0.986$, 0.753). There was no significant difference between various age and BMI subgroups in terms of selenium and 25-hydroxy vitamin D concentrations, but serum 25-hydroxy vitamin D was significantly higher in males (24.0 versus 16.4, $P < 0.001$). Moreover, serum selenium was significantly lower among patients who needed a ventilator (83.7 versus 92.8, $P = 0.02$) and those who did not survive (76.4 versus 93.5, $P < 0.001$). No significant difference was found in the serum concentrations of 25-hydroxy vitamin D between surviving and non-surviving patients (20.4 versus 21.3, $P = 0.83$). Selenium and 25-hydroxy vitamin D concentrations were not significantly different in subgroups with respect to ICU admission, loss of consciousness, and gastrointestinal and neurological symptoms. Serum 25-hydroxy vitamin D concentration was notably associated with fever; the mean of 25-hydroxy vitamin D was reported 13.2 ± 7.9 in patients with fever versus 21.8 ± 12.5 in those without fever ($P = 0.01$, (Table 2).

Figure 1: Number of patients who needed ventilation, lost consciousness, and were admitted to ICU.

Out of all patients, 13 cases were admitted to ICU. Out of 13 patients who were admitted to ICU, 8 patients needed ventilation, and all were unconscious. Thirteen patients needed ventilation which 8 of them were admitted to ICU and were unconscious.

Abbreviations: ICU: Intensive Care Unit

Pearson correlation showed a negative significant correlation between length of hospital stay ($r = -0.43$, $P < 0.001$), percentage of lung involvement ($r = -0.20$, $P = 0.04$), and serum 25-hydroxy vitamin D concentration. Inverse correlations were also observed between CRP ($r = -0.19$, $P = 0.06$), ESR ($r = -0.19$, $P = 0.06$), and 25-hydroxy vitamin D concentration, but the aforementioned correlations were not statistically significant. Besides, our findings revealed a significant negative correlation between the length of hospital stay and the serum selenium concentration ($r = -0.43$, $P < 0.001$) and a significant positive correlation between the percentage of SpO2 and serum selenium concentration ($r = -0.20$, $P = 0.04$). In addition, a negative correlation was observed between percentage of lung involvement and serum selenium concentration, which was not statistically significant ($r = -0.18$, $P = 0.07$) (Figure 2).

Results of linear regression modeling regarding the association of 25-hydroxy vitamin D and selenium concentartions with the length of hospital stay showed that after adjustment for age, gender, BMI, CRP, percentage of lung involvement, percentage of SpO2, and dietary intake of vitamin D and selenium, both serum 25-hydroxy vitamin D (coefficient = -0.10, $P = 0.006$) and selenium concentrations (coefficient = -0.09, $P = 0.01$) were significantly associated with the length of hospital stay. Each unit increase of serum 25-hydroxy vitamin D decreases the length of hospital stay by 0.1 days. Based on the standardized coefficients of the linear regression model, 25-hydroxy vitamin D showed the strongest association with the length of hospital stay (Standardized coefficient = -0.27, P-value = 0.006) (Table 3). Also, based on logistic regression modeling analysis, selenium concentration was significantly associated with outcomes of patients; each unit increase (1 ng/dl)

Variables	Number	Minimum	Maximum	Mean	SD
BMI [1] (kg/m^2)	100	17.6	37.2	26.4	4
Age (year)	100	18	65	47.2	11.1
Serum 25-hydroxy Vitamin D (ng/mL)	100	5	58.4	20.5	12.2
Serum Selenium (ng/mL)	100	31	116.5	91.8	11.8
SpO2 [2] (%)	100	65	93	87.1	6.9
Serum Magnesium (mg/dL)	100	1.6	3.1	2.2	0.3
Serum Calcium (mg/dL)	100	7	11.2	8.7	0.8
Serum Potassium (mmol/L)	100	3.1	138	5.9	13.4
Serum Sodium (mEq/L)	100	3.8	161	135.5	19.4
CRP [3] (mg/L)	100	2	89	19.5	19.3
ESR [4] (mm/hr)	100	2	88	28	20.7
Hemoglobin (g/dL)	100	8.6	21	14.5	2.2
Hematocrit (%)	100	31.2	69.2	45.9	8.5
RBC [5] ($x10^6/\mu L$)	100	3.3	80.6	6.1	7.6
MCV [6] (fL)	100	26.2	230	83.9	17.5
Platelet ($x10^3/\mu L$)	100	34.8	806	224.6	105.3
WBC [7] ($x10^9/L$)	100	1.8	103	8.2	10
Neutrophil (%)	100	5.9	93.8	70.9	15.8
Lymphocyte (%)	87	2.3	99.2	20.7	13.9
Energy (kcal)	100	1758.4	3123.1	2344.1	389.9
Protein (g)	100	88.4	166.8	120.8	21
Carbohydrate (g)	100	241.8	440.1	331.9	57.9
Total fat (g)	100	50	96	65	10.2
Cholesterol (mg)	100	294.4	633.6	439.4	79.9
Dietary Vitamin D (micg/d)	100	1.2	3.5	2.4	0.4
Dietary Selenium (mg/d)	100	75.6	164.4	113.6	24.4

Table 1: Baseline clinical and nutritional characteristics in hospitalized COVID-19 patients.

Values reported as mean ± standard deviation (SD). The blood samples were drawn in the first day of admission to hospital. The dietary vitamin D is sum of dietary vitamin D2 and D3.

Abbreviations: BMI: Body Mass Index; SpO2: Saturation of Oxygen; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; WBC: White Blood Cell

Variable	Subgroup(n)	25-hydroxy vitamin D (ng/ml)			Selenium (ng/ml)		
		Mean	SD	P-value*	Mean	SD	P-value*
Age (years)	Less than 50 (56)	19.41	11.22	0.35	91.29	12.16	0.64
	50 and above (44)	21.73	13.32		92.42	11.41	
Gender	Male (54)	23.98	13.98	0.001	92.39	11.19	0.60
	Female (46)	16.41	8.25		91.16	12.51	
BMI [1] ($kg/m^{2)}$	Normal (39)	24.17	15.00	0.05	92.39	11.62	0.26
	Overweight (47)	18.00	9.37		92.77	9.39	
	Obese (14)	18.63	10.35		87.04	17.88	
Final outcome (Death)	Survived (90)	20.41	11.72	0.83	93.53	9.30	<0.001
	None-survived (10)	21.30	17.04		76.44	19.44	
Fever (°C)	No (58)	21.79	12.47	0.01	92.93	9.68	0.16
	Yes (42)	13.19	7.80		85.53	19.19	

Ventilator need	No (87)	21.27	12.28	0.07	92.83	9.67	0.02
	Yes (13)	14.25	10.48		83.71	21.61	
ICU admission	No (87)	21.17	12.11	0.15	93.12	9.90	0.08
	Yes (13)	15.97	12.70		83.12	18.61	
Loss of consciousness	No (92)	20.76	12.15	0.46	92.30	11.75	0.17
	Yes (8)	17.44	13.82		86.36	11.39	
GI.[2] Symptoms	No (79)	21.25	12.30	0.23	93.23	9.74	0.09
	Yes (21)	17.65	11.88		86.54	16.70	
N.[3] Symptoms	No (51)	21.60	11.36	0.36	91.93	10.59	0.93
	Yes (49)	19.35	13.12		91.71	13.00	

Table 2: Comparison of 25-hydroxy vitamin D and selenium concentrations in subgroup of variables in hospitalized COVID-19 patients.

Values reported as mean ± standard deviation (SD), P-values numbers marked in bold indicate numbers that are significant (P-value: <0.05). The fever is defined as body temperature ≥ 37°C.

Abbreviations: Body Mass Index (Normal: 18.5-24.9, Overweight: 25-29.9, Obese: 30≤), Gastrointestinal symptoms (nausea, vomiting, diarrhea, constipation, and abdominal cramps), Neural symptoms (headache, anosmia, ageusia

* between group p-value (Independent T-test.

Figure 2: Pearson correlation between quantitative variables and serum concentration of 25-hydroxy vitamin D and selenium.

Pearson correlation showed a negative significant correlation between length of hospital stay (r = -0.43, P < 0.001), percentage of lung involvement (r = -0.20, P = 0.04), and serum 25-hydroxy vitamin D concentration. Our findings revealed a significant negative correlation between the length of hospital stay and the serum selenium concentration (r = -0.43, P < 0.001), and a significant positive correlation between the percentage of SpO2 and serum selenium concentration (r = -0.20, P = 0.04).

Abbreviations: So2: Saturation of Oxygen; So2.dis: Discharge Saturation of Oxygen; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; RR: Respiratory Rate; BMI: Body Mass Index

Variable	Coefficient	Standard error	Standardized coefficient	P-value
Female gender	-0.19	1.73	-0.02	0.91
Age (years)	0.03	0.04	0.08	0.36
BMI [1]	0.01	0.10	0.01	0.94
Percentage of lung involvement	0.04	0.03	0.15	0.17
Percentage of SpO2 [2]	-0.09	0.07	-0.14	0.22
CRP	0.04	0.02	0.17	0.07
25-hydroxy vitamin D (ng/ml)	-0.10	0.04	-0.27	0.006
Selenium (ng/ml)	-0.09	0.04	-0.23	0.01
Dietary Vitamin D intake (micg/d)	-0.85	1.05	-0.08	0.42
Dietary Selenium intake (mg/d)	-0.01	0.04	-0.03	0.88

Table 3: Results of linear regression for modeling effect of 25-hydroxy vitamin D and selenium concentration on length of hospital stay in hospitalized COVID-19 patients.

P-values numbers marked in bold indicate numbers that are significant (p-value: <0.05).

Abbreviations: BMI: Body Mass Index; SpO_2: Saturation of Oxygen

in serum selenium concentration caused a 15% decrease in odds of death. Moreover, each one-year increase in age led to an 18% increase in odds of death, meaning that the group of non-survivors was significantly older than survivors (Table 4).

Variable	Coefficient	Odds ratio	95% CI	P-value
Female gender	0.51	1.68	0.16, 17.68	0.68
Age (year)	0.16	1.18	1.01, 1.38	0.04
BMI [1]	-0.10	0.90	0.68, 1.19	0.47
Percentage of lung involvement	-0.01	0.99	0.92, 1.07	0.78
Percentage of SpO2 [2]	-0.09	0.92	0.78, 1.07	0.27
CRP	-0.006	0.99	0.93, 1.06	0.86
25-hydroxy vitamin D (ng/ml)	0.06	1.06	0.97, 1.16	0.21
Selenium (ng/ml)	-0.17	0.85	0.75, 0.96	0.01
Dietary Vitamin D intake (micg/d)	-0.36	0.70	0.03, 14.30	0.82
Dietary Selenium intake (mg/d)	0.08	1.08	0.93, 1.25	0.29

Table 4: Results of logistic regression for modeling effect of 25-hydroxy vitamin D and selenium concentration on death in hospitalized COVID-19 patients.

P-values numbers marked in bold indicate numbers that are significant (p-value: <0.05).

Abbreviations: BMI: Body Mass Index; SpO_2: Saturation of Oxygen

Discussion

In this study, we assessed the concentration of serum selenium and 25-hydroxy vitamin D in 100 patients with COVID-19 and the association of these micronutrients with COVID-19 outcomes, including mortality, length of hospital stay, and SpO2. The results confirmed our hypothesis that there is an inverse association between serum concentrations of selenium and 25-hydroxy vitamin D and occurrence of adverse clinical outcomes and mortality in hospitalized patients with severe COVID-19. Based on the results, 25-hydroxy vitamin D and selenium were both associated with decreased length of hospital stay. Although higher concentrations of serum selenium were associated with decreased risk of death and higher SpO2, no significant correlation was found between serum 25-hydroxy vitamin D and mortality, and SpO2. No significant associations were found between 25-hydroxy vitamin D and selenium concentrations and CRP concentration. A higher concentration of 25-hydroxy vitamin D was associated with lower occurrence of fever and lung involvement percentage; however, there was no such association with selenium concentration. Based on our results, regarding length of hospital stay and mortality, after adjusting for the effect of age, gender, BMI, CRP, percentage of lung involvement and SpO2, and dietary intake of vitamin D and selenium, 25-hydroxy vitamin D had the strongest association with the length of hospital stay and selenium had the strongest correlation with mortality. No significant association was found between 25-hydroxy vitamin D and selenium concentrations and CRP concentration.

The mean dietary intake of selenium (113.6 micg) in patients was above recommended daily allowance (RDA) as stipulated by NIH, but lower for vitamin D (2.4 micg) [53,54]. Patients consumed no selenium supplement during the study, but a daily oral dose of 1000 IU vitamin D was taken by all patients. Since the dosage was similar for all patients and blood samples were taken before the onset of supplementation, it could not affect our results and did not need to be adjusted in the models.

Clinical data on the mean concentrations of serum selenium and its association with clinical outcomes of patients with COVID-19 are mainly concerned with survival rather than other outcomes, and there is little evidence in this regard [43,45-47]. In this study, higher concentrations of serum selenium notably decreased the risk of mortality and length of hospital stay and increased the admission SpO2. No significant association was found between selenium concentration and fever, need for ventilation, admission to ICU, loss of consciousness, gastrointestinal and neural symptoms, CRP and ESR concentrations and lung involvement.

Hackler., *et al.* [45], Heller., *et al.* [45]. and Moghaddam., *et al.* [43]. investigated the effect of selenium and selenoprotein (transporter of selenium) on COVID-19 patients' survival and concluded that the concentration of selenium was higher in survivors in contrast to non-survivors and selenoprotein could predict their survival. Also, selenium deficiency among COVID-19 patients was reported to be only 2% based on our findings, which was not the same as the results of study by Im., *et al.* (42% selenium deficiency) [46]. Majeed., *et al.* [47]. also reported significantly lower concentration of selenium among COVID-19 patients in comparison to healthy controls. The only study on the length of hospital stay (Pincemail., *et al.* [55].) showed that the concentration of selenium was higher in those with a long stay (more than 10 days) versus those who stayed for a short time. In this study, after adjustment of confounding variables (age, gender, percentage of lung involvement, percentage of SpO2, and dietary intake of vitamin D and selenium), each unit increase (1ng/dl) in serum selenium concentration and a decrease in age by year caused a 15% and 18% decrease in the odds of death, respectively. Our results on the relationship of selenium with mortality are consistent with previous studies. However, to the best of our knowledge, no study has evaluated the association of selenium with SpO2, CRP, ESR, need for a ventilator, admission to ICU, loss of consciousness, and gastrointestinal and neural symptoms.

Immune and inflammatory response are interconnected as the activation of the immune system is along with production of pro-Inflammatory Interleukins (IL) and cytokine storm, causing

inflammation and oxidative stress, which results in tissue injury [56]. Also, the inflammatory response plays a vital role in clinical manifestations of COVID-19 [32]. Moreover, inflammation and coagulation are intertwined [57,58]. Blood coagulation disorders in patients with COVID-19 leads to production of micro-clots, which is a significant cause of death [59]. Previous studies showed that lower concentrations of serum selenium have been associated with poorer protective response to RNA virus infection in human studies, and in animal and cell culture models [60,61]. The effect of selenium on COVID-19 patients' outcomes and mortality is attributed to its antioxidant and anti-inflammatory properties. Also, its effect on the immune system as selenoproteins, such as selenoprotein K, plays a role in protection against viruses, along with antioxidant selenoproteins like glutathione reductase [61].

Optimal response of the immune system is highly dependent on a balanced diet and adequate intake of micronutrients, including selenium [21,23,62]. Selenium can affect both innate (macrophages) and adaptive (T and B cells) immunity. It induces a switch in macrophages phenotype from a pro-inflammatory type (M1) to an activated anti-inflammatory type (M2) [62]. Also, selenium can enhance the immune system by increasing the proliferation of NK cells and T lymphocytes, Interlukine-2 (IL-2) cytokine secretion and mitogenic lymphocyte responses, especially in combination with vitamin D [31,59,63].

The role of selenium in changing the molecular pathways involved in stress response, inflammation and poor prognosis in diseases is associated with insufficient production of selenoproteins, such as Glutathione peroxidase 1 (GPX1), GPX4, Thioredoxin Reductase 1 (TXNRD1), and selenoprotein F, K, and S (SELENO F, K, S) [31,60,64,65]. These proteins are critical in oxidative stress response as well as inhibition of nuclear factor kappa-B (NF-κB) of activated B cells. The inhibition of the NF-κB signaling pathway leads to a decline in the production of inflammatory cytokines, such as IL-6 [31,64,65].

Lastly, selenium can decrease atherogenesis in patients [59] since sodium selenite inhibits the formation of coagulation factors [66] and selenium increases the level of paraoxonase (POX1), which has been found to have anti-inflammatory and anti-atherogenic properties by preventing the formation of oxidized-low density lipoprotein (LDL) [67].

In this study, a higher concentration of serum 25-hydroxy vitamin D was significantly associated with lower incidence of fever, decreased length of hospital stay, and percentage of lung involvement, whereas no notable association was found between the concentration of 25-hydroxy vitamin D and mortality, CRP, ESR, need for a ventilator, admission to ICU, loss of consciousness, admission SpO2 and gastrointestinal and neural symptoms.

Data on the association of vitamin D with mortality are insufficient and controversial [68-70]. As to randomized controlled trials (RCTs) testing the effect of vitamin D on COVID-19 mortality and outcomes, Annweiler C., *et al*. [39] reported that daily vitamin D3 supplementation significantly improved the three-month survival of geriatric COVID-19 patients. Lakkireddy M., *et al*. [40]. showed that high doses of oral vitamin D therapy could reduce inflammatory markers including CRP in COVID-19 patients. However, Entrenas Castillo M., *et al.* [71] showed that high dose of Calcifediol (25-hydroxycholecalciferol) therapy in COVID-19 patients had no effect on concentration of serum CRP but decreased the rate of ICU admission significantly. As to observational studies, while some studies showed that there was a significant inverse correlation between vitamin D and mortality [70,72-74], some studies declared there was no notable association [75,76]. An analysis on 656 inpatient participants from the United Kingdom biobank showed no association between serum concentration of vitamin D and severity of COVID-19 patients and their mortality and more RCTs are needed [77]. Previous studies reported varying prevalence of vitamin D deficiency (≤20 ng/ml) in COVID-19 patients. Szeto., *et al.* [76]. reported 37.6% (< 20ng/ml), Im., *et al*. 76% [46] (≤20ng/dl), and Luo., *et al*. 65.1% [78] (≤12 ng/ml) vitamin D deficiency among COVID-19 patients. These controversies emphasize the importance of conducting more observational and RCT studies. Based on our results, 37% of patients had 25-hydroxy vitamin D deficiency (≤12 ng/ml). To compare with some of the previous studies, if deficiency measures ≤20 ng/ml, the deficiency rate among patients was 57%. The prevalence of vitamin D deficiency (< 20 ng/ml) in the Iranian healthy population was reported to be 56% [79]. Hence, the prevalence of vitamin D deficiency among COVID-19 patients in our study is similar to the total population. The significantly lower vitamin D concentration in Iranian women rather than men in our study can be explained by their reduced exposure to the sun because of their dressing habits [79]. After adjustment of confounding variables (age, gender, BMI, percentage of lung involvement, percentage of SpO2, and dietary intake of vitamin D and selenium), 25-hydroxy vitamin D was the most important variable associated with the length of hospital stay and each unit (1 ng/ml) increase in serum 25-hydroxy vitamin D decreased the length of hospital stay by 0.11 days. Our results were in line with other studies indicating that vitamin D deficiency is significantly associated with severity of COVID-19 (length of hospital stay and severe lung involvement) [80,81], however, we did not find any significant correlation with the risk of mortality.

Previous studies showed that vitamin D could reduce the risk of infection and was essential for both innate and adaptive immunity [30,82,83]. It has the potential to decrease inflammation (by suppressing NF-κB and interferons) without reducing antiviral activity of the immune system and viral clearance in the airway epithelial cells infected with respiratory syncytial virus, including

SARS-CoV-2 [84,85]. Vitamin D enhances the activities of the macrophages and monocytes, and modulates the function of NK cells, T and B cells and cytokine production through different molecular pathways [82].

Vitamin D can regulate the innate immune system via improvement of the phagocytic ability in immune cells [86]. Macrophages and monocytes express vitamin D receptor (VDR) and then vitamin D binds to this receptor, which substantially increases the production of antimicrobial peptides such as defensin β2 and cathelicidin antimicrobial peptide (CAMP). Also, regarding innate immune cells, after the binding of vitamin D to VDR on the macrophages and monocytes, it could improve their chemotaxis, autophagy, and phagolysosomal fusion, and therefore enhances the innate immune function against infections [30,86,87]. Furthermore, vitamin D influences adaptive immune cells by suppressing T helper 1 (Th1) and Th17, and inducing Th2. Th1 produces pro-inflammatory cytokines like IL-6, Interferon γ (IFN-γ) and Tissue Necrosis Factor (TNF-β), and Th17 produces IL17, an inflammatory interleukin, [30,86]. while Th2 produces non-inflammatory cytokines like IL-4, IL-5, IL-10, and IL-13 [30,86]. Thus, one of the effects of vitamin D on COVID-19 could be through modulating different T helper (Th) cells, which in turn decreases the oxidative stress during the active period of coronavirus contamination. Overall, vitamin D can reduce the inflammatory response and cytokine storm while improving the efficacy of the immune system, based on previous literature [30,86,87]. however, there is lack of evidences on these associations in COVID-19 patients; more studies on the association of vitamin D with inflammation and immune system in COVID-19 patients are needed.

The strength of this study is the parallel assessment of serum selenium and 25-hydroxy vitamin D and investigation of their relationship with clinical outcomes in 100 hospitalized COVID-19 patients in a prospective study. To the best of our knowledge, it is the first study to evaluate the association of selenium with SpO2, CRP, and ICU admission. Also, this study is the first one that assesses the dietary intake of micro- and macro-nutrients in patients and adjusts the effect of them on the outcomes. In addition, working with COVID-19 patients and interviewing them is challenging due to the extremely contagious nature of the disease. A relatively limited number of patients and lack of a control group are considered as limitations. Conducting large multi-center cross sectional studies with higher sample sizes and clinical trials are recommended in order to discover the effect of selenium and vitamin D on COVID-19 patients and their outcomes. One of the limitations to this study is that dietary selenium intake might not be an exact prediction of selenium status. The food recall cannot indicate the amount of selenium intake precisely since the selenium content of foods is dependent on that of agricultural soil and water. Moreover, lack of adjustment of medications and assessment of the diet quality are among limitations of this study that we recommend being considered in future studies.

Conclusion

In conclusion, higher concentration of selenium was associated with SpO2 when admitting to the hospital and higher concentration of 25-hydroxy vitamin D was associated with decreased percentage of lung involvement. Age, selenium level, and 25-hydroxy vitamin D concentration were significantly correlated with length of hospital stay. The risk of mortality in COVID-19 patients was associated with the serum concentrations of selenium and age, while no correlation was found regarding 25-hydroxy vitamin D. To sum it up, 25-hydroxy vitamin D and selenium supplementation might be useful in reducing the length of COVID-19.

Acknowledgments

The authors have no acknowledgments to declare.

Bibliography

1. Hu B., *et al*. "The cytokine storm and COVID-19". *Journal of Medical Virology* 93.1 (2021): 250-256.
2. Ludwig S and Zarbock A. "Coronaviruses and SARS-CoV-2: A Brief Overview". *Anesthesia and Analgesia* 131.1 (2020): 93-96.
3. Almutairi N and Schwartz RA. "COVID-19 with dermatologic manifestations and implications: An unfolding conundrum". *Dermatologic Therapy* 33.5 (2020): e13544.
4. Banerjee D and Rai M. "Social isolation in Covid-19: The impact of loneliness". *The International Journal of Social Psychiatry* 66.6 (2020): 525-527.
5. Chang WT., *et al*. "Cardiac Involvement of COVID-19: A Comprehensive Review". *The American Journal of the Medical Sciences* 361.1 (2021): 14-22.
6. Daneshgaran G., *et al*. "Cutaneous Manifestations of COVID-19: An Evidence-Based Review". *American Journal of Clinical Dermatology* 21.5 (2020): 627-639.
7. Dani M., *et al*. "Autonomic dysfunction in 'long COVID': rationale, physiology and management strategies". *Clinical Medicine (London, England)* 21.1 (2021): e63-e67.
8. Flanagan EW., *et al*. "The Impact of COVID-19 Stay-At-Home Orders on Health Behaviors in Adults". *Obesity (Silver Spring, Md)* 29.2 (2021): 438-445.
9. Hawkins M., *et al*. "A rapid review of the pathoetiology, presentation, and management of delirium in adults with COVID-19". *Journal of Psychosomatic Research* 141 (2021): 110350.
10. Hunt RH., *et al*. "COVID-19 and Gastrointestinal Disease: Implications for the Gastroenterologist". *Digestive Diseases (Basel, Switzerland)* 39.2 (2021): 119-139.

11. Jakovljevic M., *et al*. "COVID-19 Pandemia and Public and Global Mental Health from the Perspective of Global Health Securit". *Psychiatria Danubina* 32.1 (2020): 6-14.

12. Li Y and Xiao SY. "Hepatic involvement in COVID-19 patients: Pathology, pathogenesis, and clinical implications". *Journal of Medical Virology* 92.9 (2020): 1491-1494.

13. Post A., *et al*. "Kidney Infarction in Patients With COVID-19". *American Journal of Kidney Diseases: the Official Journal of the National Kidney Foundation* 76.3 (2020): 431-435.

14. Xiong J., *et al*. "Impact of COVID-19 pandemic on mental health in the general population: A systematic review". *Journal of Affective Disorders* 277 (2020): 55-64.

15. Nogueira-de-Almeida CA., *et al*. "COVID-19 and obesity in childhood and adolescence: a clinical review". *Jornal de Pediatria* 96.5 (2020): 546-558.

16. Asselah T., *et al*. "COVID-19: Discovery, diagnostics and drug development". *Journal of Hepatology* 74.1 (2021): 168-184.

17. Cheng VC., *et al*. "The role of community-wide wearing of face mask for control of coronavirus disease 2019 (COVID-19) epidemic due to SARS-CoV-2". *The Journal of Infection* 81.1 (2020): 107-114.

18. Lotfi M., *et al*. "COVID-19: Transmission, prevention, and potential therapeutic opportunities". *Clinica Chimica Acta; International Journal of Clinical Chemistry* 508 (2020): 254-266.

19. Przekwas A and Chen Z. "Washing hands and the face may reduce COVID-19 infection". *Medical Hypotheses* 144 (2020): 110261.

20. Noh JY., *et al*. "SARS-CoV-2 mutations, vaccines, and immunity: implication of variants of concern". *Signal Transduction and Targeted Therapy* 6.1 (2021): 203.

21. Iddir M., *et al*. "Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis". *Nutrients* 12.6 (2020): 1562.

22. Pecora F., *et al*. "The Role of Micronutrients in Support of the Immune Response against Viral Infections". *Nutrients* 12.10 (2020): 3198.

23. Calder PC., *et al*. "Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections". *Nutrients* 1181.4 (2020): 12.

24. Chandra RK. "Nutrition and the immune system: an introduction". *The American Journal of Clinical Nutrition* 66.2 (1997): 460s-463s.

25. Childs CE., *et al*. "Diet and Immune Function". *Nutrients* 11.8 (2019).

26. Gombart AF., *et al*. "A Review of Micronutrients and the Immune System-Working in Harmony to Reduce the Risk of Infection". *Nutrients* 12 (2020).

27. Junaid K., *et al*. "Effective Immune Functions of Micronutrients against SARS-CoV-2". *Nutrients* 12.10 (2020): 2992.

28. Vaughn AR., *et al*. "Micronutrients in Atopic Dermatitis: A Systematic Review". *Journal of Alternative and Complementary Medicine (New York, NY)* 6.25 (2019): 567-577.

29. Wintergerst ES., *et al*. "Contribution of selected vitamins and trace elements to immune function". *Annals of Nutrition and Metabolism* 51.4 (2007): 301-323.

30. Bae M and Kim H. "Mini-Review on the Roles of Vitamin C, Vitamin D, and Selenium in the Immune System against COVID-19". *Molecules* 25.22 (2020): 5346.

31. Shakoor H., *et al*. "Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19?" *Maturitas* 143 (2021): 1-9.

32. Zabetakis I., *et al*. "COVID-19: The Inflammation Link and the Role of Nutrition in Potential Mitigation". *Nutrients* 12.5 (2020).

33. Ye Q., *et al*. "The pathogenesis and treatment of the `Cytokine Storm' in COVID-19". *The Journal of Infection* 80.6 (2020): 607-613.

34. Ragab D., *et al*. "The COVID-19 Cytokine Storm; What We Know So Far". *Frontiers in Immunology* 11 (2020): 1446.

35. Soy M., *et al*. "Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment". *Clinical Rheumatology* 39.7 (2020): 2085-2094.

36. Jiang Q. "Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy". *Free Radical Biology and Medicine* 72 (2014): 76-90.

37. Mrityunjaya M., *et al*. "Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19". *Frontiers in Immunology* 11 (2020): 570122.

38. Notz Q., *et al*. "Clinical Significance of Micronutrient Supplementation in Critically Ill COVID-19 Patients with Severe ARDS". *Nutrients* 13.6 (2021): 2113.

39. Annweiler C., *et al*. "Vitamin D supplementation prior to or during COVID-19 associated with better 3-month survival in geriatric patients: Extension phase of the GERIA-COVID study". *The Journal of Steroid Biochemistry and Molecular Biology* 213 (2021): 105958.

40. Lakkireddy M., *et al*. "Impact of daily high dose oral vitamin D therapy on the inflammatory markers in patients with COVID 19 disease". *Scientific Reports* 11.1 (2021): 10641.

41. Sabico S., *et al*. "Effects of a 2-Week 5000 IU versus 1000 IU Vitamin D3 Supplementation on Recovery of Symptoms in Patients with Mild to Moderate Covid-19: A Randomized Clinical Trial". *Nutrients* 13.7 (2021).

42. Liu N., *et al*. "Low vitamin D status is associated with coronavirus disease 2019 outcomes: a systematic review and meta-analysis". *International Journal of Infectious Diseases* 104 (2021): 58-64.

43. Moghaddam A., *et al*. "Selenium Deficiency Is Associated with Mortality Risk from COVID-19". *Nutrients* 12.7 (2020): 2098.

44. Munshi R., *et al*. "Vitamin D insufficiency as a potential culprit in critical COVID-19 patients". *Journal of Medical Virology* 93.2 (2021): 733-740.

45. Hackler J., *et al*. "Relation of Serum Copper Status to Survival in COVID-19". *Nutrients* 13.6 (2021).

46. Im JH., *et al*. "Nutritional status of patients with COVID-19". *International Journal of Infectious Diseases* 100 (2020): 390-393.

47. Majeed M., *et al*. "An exploratory study of selenium status in healthy individuals and in patients with COVID-19 in a south Indian population: The case for adequate selenium status". *Nutrition* 82 (2021): 111053.

48. Zeng HL., *et al*. "Urinary trace elements in association with disease severity and outcome in patients with COVID-19". *Environmental Research* 194 (2021): 110670.

49. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines". National Institutes of Health (2019).

50. Bomer N., *et al*. "Selenium and outcome in heart failure". *European Journal of Heart Failure* 22.8 (2020): 1415-1423.

51. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. "Dietary Reference Intakes for Vitamin C VE, Selenium, and Carotenoids". Washington (DC): National Academies Press (US); (2000).

52. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC TC, Yaktine AL, et al., editors. "Dietary Reference Intakes for Calcium and Vitamin D". Washington (DC): National Academies Press (US); (2021).

53. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional

54. https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional

55. Pincemail J., *et al*. "Oxidative Stress Status in COVID-19 Patients Hospitalized in Intensive Care Unit for Severe Pneumonia. A Pilot Study". *Antioxidants (Basel, Switzerland)* 10.2 (2022).

56. Tufan A., *et al*. "COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs". *Turkish Journal of Medical Sciences* 50.SI-1 (2020): 620-632.

57. Magro G. "COVID-19: Review on latest available drugs and therapies against SARS-CoV-2. Coagulation and inflammation cross-talking". *Virus Research* 286 (2020): 198070.

58. Paces J., *et al*. "COVID-19 and the immune system". *Physiological Research* 69.3 (2020): 379-388.

59. Kieliszek M and Lipinski B. "Selenium supplementation in the prevention of coronavirus infections (COVID-19)". *Medical Hypotheses* 143 (2020): 109878.

60. Bermano G., *et al*. "Selenium and viral infection: are there lessons for COVID-19?" *British Journal of Nutrition* 125.6 (2018): 618-627.

61. Avery JC and Hoffmann PR. "Selenium, Selenoproteins, and Immunity". *Nutrients* 10.9 (2018): 1203.

62. Pal A., *et al*. "Zinc and COVID-19: Basis of Current Clinical Trials". *Biological Trace Element Research* 199.8 (2021): 2882-2892.

63. Delesderrier E., *et al*. "Selenium Status and Hemolysis in Sickle Cell Disease Patients". *Nutrients* 11.9 (2019): 2211.

64. Braunstein M., *et al*. "Selenium and Selenoprotein P Deficiency Correlates With Complications and Adverse Outcome After Major Trauma". *Shock* 53.1 (2020): 63-70.

65. Salehi M., *et al*. "Selenium supplementation improves the nutritional status of hemodialysis patients: a randomized, double-blind, placebo-controlled trial". *Nephrology Dialysis Transplantation* 28.3 (2013): 716-723.

66. Yan D., *et al*. "Sodium Selenite Improves The Therapeutic Effect Of BMSCs Via Promoting The Proliferation And Differentiation, Thereby Promoting The Hematopoietic Factors". *Onco Targets Ther* 12.5 (2019): 968.

67. Mirmohammadsadeghi A., *et al*. "Effects of selenium supplementation on paraoxonase-1 and myeloperoxidase activity in subjects with cardiovascular disease: the Selenegene study, a double-blind randomized controlled trial". *Archives of Medical Sciences Atherosclerotic Diseases* 3 (2018): e112-e118.

68. Kazemi A., *et al*. "Association of Vitamin D Status with SARS-CoV-2 Infection or COVID-19 Severity: A Systematic Review and Meta-analysis". *Advances in Nutrition* (2021).

69. Shah K., *et al*. "Vitamin D supplementation, COVID-19 and disease severity: a meta-analysis". *QJM : Monthly Journal of the Association of Physicians* 114.3 (2021): 175-181.

70. Carpagnano GE., *et al*. "Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19". *Journal of Endocrinological Investigation* 44.4 (2021): 765-771.

71. Entrenas Castillo M., *et al*. "Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study". *The Journal of Steroid Biochemistry and Molecular Biology* 203 (2020): 105751.

72. Annweiler C., *et al*. "Vitamin D and survival in COVID-19 patients: A quasi-experimental study". *The Journal of Steroid Biochemistry and Molecular Biology* 204 (2020): 105771.

73. Radujkovic A., *et al*. "Vitamin D Deficiency and Outcome of COVID-19 Patients". *Nutrients* 12.9 (2020).

74. Ling SF., *et al*. "High-Dose Cholecalciferol Booster Therapy is Associated with a Reduced Risk of Mortality in Patients with COVID-19: A Cross-Sectional Multi-Centre Observational Study". *Nutrients* 12.12 (2020): 3799.

75. Hastie CE., *et al*. "Vitamin D and COVID-19 infection and mortality in UK Biobank". *European Journal of Nutrition* 60.1 (2021): 545-548.

76. Szeto B., *et al*. "Vitamin D Status and COVID-19 Clinical Outcomes in Hospitalized Patients". *Endocrine Research* 46.2 (2021): 66-73.

77. Hastie CE., *et al*. "Vitamin D and COVID-19 infection and mortality in UK Biobank". *European Journal of Nutrition* 60.1 (2021): 545-548.

78. Luo X., *et al*. "Vitamin D Deficiency Is Associated with COVID-19 Incidence and Disease Severity in Chinese People [corrected]". *The Journal of Nutrition* 151.1 (2021): 98-105.

79. Vatandost S., *et al*. "Prevalence of vitamin D deficiency in Iran: A systematic review and meta-analysis". *Nutrition and Health* 24.4 (2018): 269-278.

80. AlSafar H., *et al*. "COVID-19 Disease Severity and Death in Relation to Vitamin D Status among SARS-CoV-2-Positive UAE Residents". *Nutrients* 13.5 (2021): 1714.

81. Sulli A., *et al*. "Vitamin D and Lung Outcomes in Elderly COVID-19 Patients". *Nutrients* 13.3 (2020).

82. Charoenngam N and Holick MF. "Immunologic Effects of Vitamin D on Human Health and Disease". *Nutrients* 12.7 (2020): 2097.

83. Trochoutsou AI., *et al*. "Vitamin-D in the Immune System: Genomic and Non-Genomic Actions". *Mini Reviews in Medicinal Chemistry* 15.11 (2015): 953-963.

84. Alexander J., *et al*. "Early Nutritional Interventions with Zinc, Selenium and Vitamin D for Raising Anti-Viral Resistance Against Progressive COVID-19". *Nutrients* 12.8 (2020): 2358.

85. Hansdottir S., *et al*. "Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state". *The Journal of Immunology* 184.2 (2010): 965-974.

86. Sassi ., *et al*. "Vitamin D: Nutrient, Hormone, and Immunomodulator". *Nutrients* 11.10 (2018): 1656.

87. L Bishop E., *et al*. "Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory". *JBMR Plus* 5.1 (2021): e10405.