

Evaluating the Relationship Between Disease Severity and Serum Levels of Zinc, Calcium and Vitamin D in COVID-19 Patients

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Abstract

Introduction

SARS-CoV-2 is spreading rapidly worldwide these days so that it has infected people in many countries. It is a zoonotic virus and the cause of COVID-19 infectious pneumonia. The World Health Organization (WHO) declared it a pandemic on January 30, 2020. Given that no standard treatment has been found for the new coronavirus so far, the present study seeks a way to reduce the incidence and severity of the disease along with health protocols. Some of the factors possibly effective in getting less infected by the SARS-CoV-2 are taking medication supplements such as zinc, calcium, and vitamin D.

Materials and methods

This cross-sectional study was conducted from May 13 to May 30, 2020, on 93 COVID-19 patients admitted to Khatam Al-Anbia Hospital in Shushtar in southwestern Iran. Some patients' laboratory and clinical data were collected and analyzed using the Chi-squared test, the independent t-test, the Kruskal-Wallis test, and the Spearman rank-order correlation coefficient by IBM SPSS Statistics 18.0 software.

Findings

The severity of the disease (40%) of 37 patients was severe in pulmonary involvement. Serum levels of vitamin D and zinc were lower than the average in all patients. Still, the severity of COVID-19 in patients was not significantly different from their zinc serum levels ($P = 0.216$). Serum vitamin D was not significantly different ($P = 0.102$). The severity of COVID-19 in patients was significantly different according to serum calcium levels ($P = 0.005$). The lower the calcium level, the more severe the disease.

Conclusion

Given that the supplementation's effect in preventing COVID-19 has not been confirmed and no study has been published on the appropriate dose of these supplements in COVID-19, taking economically viable calcium-rich food sources, including dairy, is recommended.

Introduction

The SARS-CoV-2 is spreading rapidly worldwide, so that it has infected many countries. It is a zoonotic virus and the causative agent of COVID-19 pneumonia, which the WHO identified as a pandemic (1). The SARS-CoV-2 causes much lower mortality in patients than SARS-CoV and MERS-CoV, but has a significant potential for infection and transmission (2).

The WHO declared COVID-19 to be an epidemic because it is still spreading rapidly worldwide (3). Currently, the COVID-19 pandemic is one of Iran's most important health issues and the world's (4). The SARS-CoV-2 can be transmitted from human to human, and no effective treatment has been found so far. The most effective measures are prevention and control, finding suspicious patients and close contact,

confirming patients and virus carriers, and preventing transmission through isolation, disinfection, and personal protection (2). Given that standard treatment for the COVID-19 has not yet been found (5), the present study seeks to reduce this disease's incidence and severity along with health protocols. A factor that may be effective in this way is taking supplements such as vitamin D, calcium, and zinc.

One of these elements is zinc, which is of particular importance due to the global epidemic of COVID-19. Zinc is considered a potential supportive treatment in treating COVID-19 infection due to its immunomodulatory effect and direct antiviral effect (6).

Zinc ion has been shown to directly inhibit SARS-CoV-1 RNA-dependent RNA polymerase activity from suppressing viral genomic RNA replication and preventing viral particle proliferation as a mechanism of action (7). Not only does it reduce the number of lymphocytes, but it also impairs the function of T and B lymphocytes (8, 9). Earlier studies demonstrated that a decreased zinc level favors this interaction of ACE2 with SARS-CoV-2 spike protein and likewise that an increased zinc level inhibits ACE2 expression resulting in reduced viral interaction (10).

Calcium is one of the most abundant minerals in the body, which makes up 39% of the body's total salts. Calcium plays a vital role in the immune system. It is very effective in nerve conduction, blood clotting, heart rate regulation, secretion of hormones and enzymes, and muscle contraction (11, 12). In addition to playing an essential role in strengthening bones and teeth, as a messenger, calcium also plays a significant role in cellular signaling pathways throughout the body (13, 14). Another element is vitamin D, which dates back hundreds of years to the link between vitamin D deficiency and infectious diseases. Nineteenth-century studies found that sunlight was beneficial for patients suffering from tuberculosis (15).

Vitamin D plays an influential role in autoimmune diseases, cardiovascular disease, allergic and respiratory disorders (16, 17) This vitamin has immune regulating properties. Acting on the immune system inhibits the production of inflammatory cytokines and stimulates the production of antimicrobial peptides. This peptide plays an essential role in the body's defense against respiratory pathogens, as it both strengthens the immune response and clears bacteria from defense barriers and immune cells (18). Vitamin D deficiency also increases the risk of viral infections such as RSV-induced bronchiolitis and seasonal influenza (19). Numerous studies have shown an association between vitamin D and respiratory asthma symptoms. This connection is probably through the effect of vitamin D on immune system regulation (20).

This vitamin also plays an essential role in regulating the immune system through the interaction between 1 and 22-dihydroxyvitamin D and the vitamin D 1 receptor (VDR) (21).

In "A Review on the Role of Vitamin D in Asthma", Adite et al. (2009) found that it reduces respiratory infections, prevents asthma attacks, steroid resistance, reduces osteoporosis, and controls asthma. It becomes chronic and also plays an immune role in increasing the antimicrobial peptide and the production of interleukin-10 (22) Studies on the association of serum levels of vitamin D, calcium, and

zinc with the severity of COVID-19 have yielded conflicting results. The results of some studies show that serum levels of vitamin D affect the COVID-19 severity, although studies in some areas do not confirm this relationship. This study intends to investigate the relationship between disease severity and serum levels of zinc, vitamin D, and calcium in COVID-19 patients.

Materials And Methods

Subjects and data collection

This cross-sectional study was conducted in 2020 to evaluate the serum levels of vitamin D, calcium, and zinc on the severity of the disease in patients with COVID-19, after obtaining the necessary permits from Shoushtar University of Medical Sciences. After receiving the required permission to collect data, the researcher went to the hospital and performed sampling by explaining the research objectives, obtaining patient satisfaction, and obtaining informed consent from the subjects.

This study was conducted from May 13 to May 30, 2020, on 93 COVID-19 patients admitted to Khatam al-Anbia Hospital in Shoushtar in southwestern Iran. For SARS-CoV-2 nucleic acid detection, the real-time reverse-transcriptase-polymerase-reaction (RT-PCR) was performed on nasopharyngeal and oropharyngeal swabs.

The subjects were selected via a purposive sampling method among COVID-19 patients admitted to Shoushtar Khatam Al-Anbia Hospital. Blood samples were taken from the patients by a hospital nurse (a project colleague) and sent to a private laboratory for more investigation.

Inclusion criteria were as follows: 1. having an Iranian ethnicity and being a resident of Shoushtar city; 2. being infected with COVID-19 based on PCR test; 3. having the willingness to participate in the study; 4. being over 15 years old, and 5. being fasting during sampling.

Exclusion criteria were as follows: 1- living outside of Shoushtar city 2- having dissatisfaction to participate in the study; 3- taking Vitamin D, calcium, and zinc supplements during the last six months before the study; 4- taking corticosteroid drugs, cholesterol-lowering drugs such as cholesterol and barbiturates and phenytoin (which they can reduce the serum levels of vitamin D, calcium and zinc in the body); 5. having parathyroid disease, bone disease, chronic liver disease, kidney disease, and cancer; and 6. having been treated with vitamin D, calcium and zinc.

Laboratory and clinical results

In this study, we measured serum levels of vitamin D, calcium, and zinc. Serum vitamin D levels were measured by ELISA (Enzyme-Linked Immunosorbent Assay). Serum levels of vitamin D were divided into 30ng/ml-100 ng/ml as sufficient, 10 ng/ml -30 ng/ml as insufficient, and < 10 ng/ml as deficient. Calcium Arsenazo measured serum levels of calcium with a reference range of 8.6–10.3 mg/dl. Serum levels of

zinc were measured by Atomic Absorption Spectrophotometry (AAS) with a reference range of 70–127 µg/dl.

In this study, information such as age, gender, place of residence, calcium, zinc, and vitamin D supplementation, history of underlying diseases (diabetes, hypertension, heart disease, lung disease, hyperlipidemia); symptoms and possible complications of the disease (cough, shortness of breath). Stenosis, fever, muscle weakness, pain, nausea, vomiting, headache, dizziness, abdominal pain, seizures, chest pain, palpitations, blurred vision, diarrhea, restlessness, aggression, decreased sense of smell, etc.). Laboratory results such as serum levels of vitamin D, calcium, zinc, blood urea nitrogen (BUN), creatinine (cr), sodium (Na), potassium (K), white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HEMA), platelet (PLT), lymphocyte (Lym), neutrophils (Neut), Arterial Blood Gas Test (PH, PO₂, PCO₂, HCO₃), erythrocyte sedimentation rate (ESR), and blood sugar (BS) were recorded.

Statistical analysis

According to the research objectives, previous studies (55), and the parameter of respiratory problems in COVID-19 patients in the two normal serum calcium and low serum calcium groups, the subjects' proportions with respiratory problems in the two groups were $P_1 = 0.06$ and $P = 0.016$. Also, given $\beta = 0.9$, $\alpha = 0.05$, $d = 0.08$ and using the sample volume ratio formula, the case group was estimated to consist of 93 subjects.

Blood test results, demographics, and clinical information were entered into IBM SPSS Statistics 18.0 software. Quantitative variables are reported as mean (average), standard deviation (mid-quarter range), minimum and maximum, and qualitative variables are reported as number (percentage). The Kolmogorov-Smirnov test was employed to confirm the normality of quantitative variables, the Spearman rank-order correlation coefficient to examine the relationship between quantitative variables, the independent t-test to compare means, the Kruskal-Wallis test to compare the levels of variables, the Chi-squared test to compare qualitative variables in terms of number, and sequential regression to predict some factors affecting the severity of COVID-19. The significance level of the above tests is considered less than 0.05.

Findings

The median [interquartile range, (IQR)] age of the 97 subjects was 51 (40–61). Fifty-two subjects (55.9%) were female. The median [interquartile range, (IQR)] length of hospital stay was four days (3–5). Thirty-seven subjects (40%) suffered from severe pulmonary involvement.

Table 1
Comparison of underlying diseases and symptoms at different disease severity levels

p-value chi-square	The disease severity					
	N(%)					
	SEVER N = 37	MEDIAN N = 30	LOW N = 26			
0.06	10(66.7%)	2(13.3%)	3(20.0%)	15(16.1%)	Diabetes	Underlying diseases
0.292	6(60.0%)	3(30.0%)	1 (10.0%)	10(10.8%)	Hypertension	
0.249	1 (12.5%)	4(50.0%)	3(37.5%)	8(8.6%)	Pulmonary diseases	
0.024	12(60.0%)	7(35.0%)	1 (5.0%)	20(21.5%)	Cardiovascular diseases	
0.039	11(25.0%)	16(36.4%)	17(38.6%)	44(47.3)	No Underlying diseases	
0.196	12(44.4%)	5(18.5%)	10(37.0%)	27(29.0%)	Fever	Disease symptoms
0.905	22(39.3%)	19(33.9%)	15(26.8%)	56(60.2%)	Cough	
0.849	16(42.1%)	11(28.9%)	11(28.9%)	38(40.9%)	Dyspnea	
0.515	6(33.3%)	5(27.8%)	7(38.9%)	18(19.4%)	Muscle pain	
0.647	3(27.3%)	4(36.4%)	4(36.4%)	11(11.8%)	Headache	
0.014	5(27.8%)	3(16.7%)	10(55.6%)	18(19.4%)	Anorexia	
0.523	4(33.3%)	3(25.0%)	5(41.7%)	12(12.9%)	Fatigue	
0.460	1 (16.7%)	3(50.0%)	2(33.3%)	6(6.5%)	Sore throat	
0.486	2(25.0%)	4(50.0%)	2(25.0%)	8(8.6%)	Digestive symptoms	
0.117	0(0.0%)	2(100.0%)	0(0.0%)	2(2.2%)	Diminished sense of smell	

The Mean \pm SD serum level of vitamin D in the subjects was 11.21 ± 21.40 with a variation range of 8–56. Sixty-eight subjects (73%) had vitamin D deficiency (< 30), and 25 (27%) of them had normal serum levels of vitamin D (30–100).

The Mean \pm SD serum level of calcium in the subjects was 9.14 ± 0.39 with a variation range of 8.40–10.30. Thirty-nine subjects (42%) had calcium deficiency (< 9), and 54 (58%) of them had normal calcium levels (9-10.5).

The Mean \pm SD serum level of zinc in the subjects was 67.61 ± 15.10 with a variation range 41–112. Forty-nine subjects (52.7%) had zinc deficiency (< 70), and 44 (47.3%) of them had normal serum levels of zinc (70–114).

Serum vitamin D levels were not statistically significant between the male and female subjects ($P = 0.182$); however, serum levels of vitamin D were higher in male than female subjects. Serum levels of calcium were not significantly different between the male and female subjects ($P = 0.059$), but in the female subjects, serum calcium levels were lower than the male subjects. Serum zinc levels were not significantly different between the male and female subjects ($P = 0.591$). The disease's severity was higher in the male than female subjects ($P = 0.009$) (See Table 2).

Table (2): Comparison of serum levels of vitamin D, calcium, zinc, and disease severity between men and women

	Male N = 41	Female N = 52	p-value t-test
ZINC (normal:70–127 μ g/dl)	66.65 ± 16.15	68.36 ± 14.33	0.591
CALCIUM (normal:8.6–10.3 mg/dl)	9.21 ± 0.41	9.05 ± 0.35	0.059
VITAMIN D (normal:30–100 ng/ml)	22.95 ± 11.20	20.25 ± 11.18	0.182
Severity of the disease	2.36 ± 0.76	1.92 ± 0.81	0.009

The severity of the disease in coronary arteries was not significantly different according to the serum level of vitamin D ($P = 0.102$). The COVID-19 severity in patients was significantly different according to serum calcium levels ($P = 0.005$). The lower the serum levels of calcium, the more severe the disease. The severity of the COVID-19 was not significantly different in the subjects in terms of their serum levels of zinc ($P = 0.216$). There was a statistically significant and direct relationship between the severity of the disease with the age of patients ($P < 0.001$) and length of hospital stay ($P = 0.026$). With increasing age, the disease severity increases. Also, with increasing the disease severity, hospitalization prolongs. There was a statistically significant and direct relationship between BS and disease severity ($P = 0.007$). The higher the blood sugar level is, the more severe the disease is. People with more severe diseases had higher blood ESR levels ($P = 0.048$) (See Tables 3&4).

Table (3): Comparison of some demographics, serum levels of vitamin D, calcium, and zinc, and laboratory results at different levels of the disease severity

		Severity of the disease			P-VALUE
		LOW	MEDIAN	SEVER	/chisquar
		N = 26	N = 30	N = 37	/ANOVA
					/ Kruskal Wallis Test
		N (%)			
Gender	Male	7(17.1%)	12(29.3%)	22(53.7%)	.032
	Female	19(36.5%)	18(34.6%)	15(28.8%)	
		Meam ± sd			
VITAMIN D		20.02 ± 10.40	19.12 ± 11.21	24.57 ± 11.42	.077
CALCIUM		9.28 ± 0.36	9.10 ± 0.43	9.08 ± 0.36	.043
ZINC		69.11 ± 15.27	69.46 ± 17.80	65.05 ± 12.42	.417
Age		42.23 ± 13.76	49.73 ± 15.44	59.16 ± 13.58	.121
Duration in hospitalization		3.76 ± 2.23	4.16 ± 1.23	4.64 ± 2.09	.050
BUN		13.35 ± 3.60	21.00 ± 18.55	18.45 ± 11.02	.301
CR		0.92 ± .18	1.15 ± .69	1.11 ± .40	.169
NA		140.47 ± 1.84	139.32 ± 4.48	136.35 ± 18.22	.344
K		6.65 ± 9.66	4.25 ± 0.30	4.46 ± .56	.236
WBC		6.96 ± 2.34	6.49 ± 3.34	6.93 ± 2.36	.814
RBC		4.65 ± .49	4.35 ± .56	4.57 ± .74	.329
HB		13.17 ± 1.42	12.41 ± 1.63	12.10 ± 1.51	.06
HEMA		39.02 ± 4.67	36.84 ± 4.33	38.10 ± 3.74	.280
PLT		232.29 ± 61.79	193.60 ± 71.01	240.18 ± 98.42	.140
LYN		27.46 ± 16.58	29.24 ± 16.69	26.75 ± 20.22	.520
NOTRO		68.07 ± 12.25	62.92 ± 15.27	69.52 ± 10.29	.174
PH		7.37 ± .04	7.37 ± .04	7.38 ± .08	.869
PCO2		38.22 ± 4.51	37.22 ± 7.09	37.29 ± 8.01	.947
PO2		35.06 ± 12.0	34.42 ± 12.69	34.57 ± 11.15	.101

	Severity of the disease			P-VALUE
	LOW N = 26	MEDIAN N = 30	SEVER N = 37	/chisquar /ANOVA / Kruskal Wallis Test
HCO3	21.55 ± 0.67	21.66 ± 2.56	22.61 ± 2.91	.554
ESR	33.89 ± 17.75	22.25 ± 12.10	47.00 ± 29.24	.048
BS	105.56 ± 36.47	140.94 ± 78.5	173.18 ± 86.67	.028

Note:(Blood Urea Nitrogen(BUN), creatinine(cr), Sodium(Na), Potassium(K), WHITE BLOOD CELL(WBC), RED BLOOD CELL(RBC), HEMOGLOBIN(HB), HEMATOCRIT(HEMA), PLATELET(PLT), LYMPHOCYTE(Lym), neutrophils(Neut), Arterial Blood Gas Test(PH,PO2,PCO2, HCO3), Erythrocyte sedimentation rate(ESR), Blood Sugar(BS).

Table (4): Investigation of the relationship between some demographics, serum levels of vitamin D, calcium, and zinc, and laboratory results at different disease severity levels

	Severity of the disease	P-VALUE
	Spearman's rho	
VITAMIN D	.175	.102
CALCIUM	-.292	.005
ZINC	-.129	.216
Age	.457	.000
BUN	.090	.390
CR	.179	.085
NA	-.052	.622
K	.025	.813
WBC	.037	.757
RBC	-.052	.666
HB	-.249	.034
HEMA	-.012	.919
PLT	.021	.864
LYN	-.109	.378
NOTRO	.058	.640
PH	.227	.246
PCO2	-.081	.681
PO2	.329	.088
HCO3	.260	.212
ESR	.213	.235
BS	.360	.007

The rank regression was employed to examine the effect of other independent variables on the dependent variable. First, all the independent variables were entered into the model one by one. Each independent variable that had a significance level lower than 0.2 was entered into the final model. Of all the independent variables, only the variables vit.d, calcium, age, hemoglobin, blood sugar had a single effect on the dependent variable (the subjects' severity of pulmonary involvement). The other variables were also entered into the model. Based on the logit = $\log(x / (1-x))$ link function, the model was valid (-2 Log-Likelihood = 159.418, Chi-Square = 35.29, df = 8, sig. <0.001). The independent variables entered in the model can explain and predict 37% of the dependent variable variation (Nagelkerke = 0.369). Variables

such as blood glucose and age have significant and direct effects, and calcium and hemoglobin variables have significant and indirect effects on the subjects' pulmonary involvement severity.

Table (5): Estimation of independent variables on disease severity by sequential regression

		Estimate	Std. Error	Wald	df	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Threshold	[Severity = low]	-12.930	5.898	4.806	1	.028	-24.490	-1.370
	[Severity = Median]	-10.892	5.847	3.470	1	.063	-22.353	.569
Location	AGE	.050	.016	9.837	1	.002	.019	.082
	Vitamin D	.024	.021	1.291	1	.256	-.017	.065
	Calcium	-1.253	.580	4.662	1	.031	-2.390	-.116
	CR	-1.193	.638	3.493	1	.062	-2.444	.058
	HB	-.472	.181	6.776	1	.009	-.828	-.117
	BS	.011	.004	3.775	1	.048	-0.00007	.016
	PO2	.065	.040	2.713	1	.100	-.012	.143
	[sex = male]	1.072	.528	4.120	1	.042	.037	2.107
	[sex = female]	0 ^a	.	.	0	.	.	.
Link function: Logit.								
a. This parameter is set to zero because it is redundant.								

Discussion

This study aimed to investigate the relationship between disease severity and serum levels of zinc, calcium, and vitamin D in COVID-19 patients. The findings show that serum levels of vitamin D and zinc did not affect the COVID-19 severity, given that serum levels are both lower than normal. However, serum calcium levels have a significant and direct effect on disease severity and may help predict the risk of more severe disease in COVID-19 patients.

Existing studies suggest that the nutrients zinc, calcium, and vitamin D may play a role in the course and severity of COVID-19 disease. However, based on SARS and other viral infections, the present research observations and results suggest nutritional supplements increase host resistance to RNA viral infections, including severe COVID-19. In this study, the severity of the disease in 37 patients (40%) was associated

with their pulmonary involvement. In Cao X et al. (2020), most COVID-19 patients showed mild to moderate symptoms, but approximately 15% suffered from severe pneumonia (23).

Moreover, Huang (2020) indicated that the disease's severity resulted in mortality (24). The present study showed that serum levels of zinc in all three groups were lower than normal. Still, the severity of the disease in the COVID-19 patients did not differ significantly according to their zinc serum levels. Yao et al. (2021) did not observe hospital survival time and disease severity (25). In an RCT study, Bansal et al. (2011) concluded that zinc supplementation does not affect severe and very severe pneumonia (26). However, Wessels et al. (2020) stated that zinc supplements are associated with shortening symptoms, reduced disease severity, and, most importantly, reduced morbidity and mortality in COVID-19 patients (27).

In the present study, serum levels of vitamin D were lower than normal in all three groups. Still, there was no significant difference in the COVID-19 severity concerning serum levels of vitamin D ($P = .014$). At the same time, in "Assessing Vitamin D Deficiency in Asthmatic Children and Its Effect on Asthma Severity", Sadghi et al. (2017) found that there was a significant inverse relationship between serum levels of vitamin D and asthma severity (31). Amrein et al. (2014) stated that vitamin D3 deficiency is one of the causes of elevated plasma glucose and dyslipidemia.

Also, the modification of the plasma level of vitamin D3 can lead to decreased insulin resistance and the modification of plasma lipid patterns in patients with type 2 diabetes (28). Radujkovic et al. (2020) stated in their study that VITD levels did not differ significantly between disease severity groups (29). Hollams et al. (2011) showed that vitamin D deficiency is associated with asthma severity in children with the disease (19). In Brehm Childcare Asthma Management (CAMP) (2010) conducted on children with asthma in North America, low levels of vitamin D were directly associated with an increased risk of hospitalization (30). Barassi (2021) showed serum levels of vitamin D in patients with severe COVID-19, and more need for artificial ventilation was low (31)

In a randomized clinical trial study of COVID-19 hospitalized patients, Murai (2021) concluded that a high dose of vitamin D3, compared with placebo, did not significantly reduce hospital stays, and the use of high doses of vitamin D3 For the treatment of COVID-19 does not confirm moderate to severe (32). Among hospitalized patients with COVID-19, a single high dose of vitamin D3, compared with placebo, did not significantly reduce hospital length of stay. The findings do not support the use of vitamin D3 for the treatment of moderate to severe COVID-19.

The results also showed that the COVID-19 severity in patients was significantly different according to serum calcium levels ($P = 0.037$). The lower the calcium level, the more severe the disease. Recently, a high prevalence of hypocalcemia in COVID-19 patients was reported in several other articles. Sun (2020) stated that serum levels of calcium were associated with disease severity and prognosis in COVID-19 patients (33).

The results of Yung et al. (2020) were consistent with those of the present study, as they reported that hypocalcemia might be an indicator for patients who are likely to have a severe disease (34). Also, the results of Sun et al. (2019) (35). and Luigi et al. (2020) (36). are consistent with those of the present study.

The results of our study showed that the severity of the disease was statistically significant and directly related to the age of patients ($P < 0.001$) and length of hospital stay ($P = .026$). The severity of the disease increases with age. Lauc et al. State that the most critical predictor of COVID-19 disease severity is age (37). Huang I et al. (2020) also named old age as one of the causes of COVID-19 mortality (26). Targher et al. (2020) described older age as an independent variable with a higher risk of severe COVID-19 (38). In Lix et al. (2020), elderly patients were associated with severe COVID-19 (39). The present study results showed that the severity of the disease is higher in male than female subjects.

In "Clinical Period and Risk Factors for Adult Mortality Patients with COVID-19 in Wuhan, China", Fei Zhou et al. (2020) showed that most of the patients were male (34). Also, in the study of Targher et al. (2020), males were among the variables that were independently associated with a higher risk of severe COVID-19 disease (38). In Lix et al. (2020), male patients were related to severe COVID-19 and death (39). While in Shamsizadeh (2019) (40), no statistically significant relationship was found between disease severity and gender.

Our study results showed that people whose disease was more severe had higher blood ESR levels. Deng SQ (2020) Most of the subjects had high ESR (2).

There was a statistically significant and direct relationship between BS and disease severity ($P = 0.007$). The higher a patient's blood sugar, the higher the severity of the disease. Targher et al. (2020) reported that diabetes is associated with an approximately 4-fold increase in the risk of severe COVID-19 (38). In Lix et al. (2020), patients with hypertension were associated with severe COVID-19 and higher mortality rates (39). Results of Deng (2020) (2), Zhang (2020) (44), Ruan (2020) (33), and Huang (2020) (24). also linked diabetes to disease severity.

Conclusion

Our findings show that serum levels of vitamin D and zinc are lower than normal in all patients and have no effect on the COVID-19 severity. However, serum levels of calcium have a significant and direct effect on disease severity and may help predict the risk of more severe disease in COVID-19 patients. Because no study confirms the supplementation to help prevent COVID-19 and no research on the appropriate dose of the supplements in COVID-19 have not been published so far, the consumption of calcium-rich food sources, such as dairy, are economically viable, is recommended.

Suggestions

Because COVID-19 is a pandemic and there is currently no definitive cure for it, we must look for ways to prevent it. It is suggested that other studies with larger sample sizes in different parts of the world

evaluate whether deficiency of these supplements is associated with an increase in the COVID-19 severity. Also, more immediate clinical trials are recommended to reach a definitive conclusion about taking vitamin D, Calcium, and zinc supplements to reduce the risk of getting infected by COVID-19, and its severity.

Abbreviations

Vit.D: Vitamin D;CA: calcium; RNA: RiboNucleic Acid; ACE2: Angiotensin-converting enzyme 2; CoV: **Coronavirus**; VDR: vitamin D 1 receptor; ELISA:Enzyme-Linked Immunosorbent Assay; AAS :Atomic Absorption Spectrophotometry ;SD: Standard deviation; IQR: Inter Quartile Range; WHO: World Health Organization ; BUN :Blood Urea Nitrogen; CR: Creatinine; Na :Sodium; K :Potassium; WBC :White Blood Cell; RBC :Red Blood Cell; HB: Hemoglobin; HEMA: Hematocrit; PLT: Platelet; LYM: Lymphocyte; Neut : Neutrophils; ABG: Arterial Blood Gas ; PH: **Power** of Hydrogen ; PO2: Partial Pressure of Oxygen; PCO2 :**Partial Pressure of Carbon Dioxide**, HCO₃ Hydrogen Carbonate; ESR: Erythrocyte Sedimentation Rate; BS: Blood Sugar.

Declarations

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Authors'contributions

Conception and designof study: A. Jahangirimehr, A. Khalighi;

Acquisition of Data: A. Khalighi, N.Bahmanyari, M.Labibzadeh;

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Drafting the Manuscript: A. Jahangirimehr, A. Khalighi, E.Abdolahishahvali;

Revising the manuscript critically for important intellectual content: A. Jahangirimehr, A. Khalighi, E.Abdolahishahvali.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request

Ethical Statement

We herewith confirm that the trial protocol has been approved by the Central Ethics Commission of the Ministry of Healthcare of IRAN .This article is from a research project entitled Serum levels of vitamin D, calcium and zinc in people with coronavirus 19 in Shoushtar city with ethics code IR.SHOUSHTAR.REC.1399.017 .A written and oral informed consent was presented to each participant prior to participating in the study.

Consent for publication

This manuscript has not been published elsewhere by another journal.

Competing interests

The authors declared no competing interests.

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