ORIGINAL ARTICLE

Serum level of Vitamin D is associated with COVID-19 mortality rate in hospitalized patients

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Background: Due to widespread of coronavirus disease 2019 (COVID-19) infection, identification of its risk factors and clinical characteristics are important. The aim of the present study was to assess Vitamin D levels in individuals with severe acute respiratory syndrome coronavirus-19 infection and to report on its potential as a predictive marker. **Materials and Methods:** All patients, diagnosed with COVID-19 infection from February 16 to March 21, 2020, and referred to Firoozgar Hospital, Tehran, Iran, were enrolled in this study. Vitamin D analysis was undertaken on patient serum samples using a commercial kit (Pars Azmoon Co., Tehran, Iran). SPSS v. 22 was used for statistical analysis. **Results:** Vitamin D serum concentration was analyzed in a total of 317 patients whose mean age ± standard deviation was 62.05 ± 15 years and with 62.5% being male. A significant association of Vitamin D level and death was observed. Higher levels of serum Vitamin D had protection against death (odds ratio = 0.955 [95% confidence interval = 0.923–0.988], $P = 0.008$). **Conclusion:** As a preliminary study in the Iranian population who suffered COVID-19 disease, we identified that Vitamin D deficiency was associated with a higher death rate and intensive care unit admission.

Key words: Coronavirus disease 2019, malnutrition, respiratory tract infection, Vitamin D

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INTRODUCTION

Novel coronavirus disease 2019 (COVID-19) is currently a worldwide health concern that causes mild upper respiratory tract infection (URTI) to severe lower respiratory tract infection (LRTI) and severe acute respiratory syndrome (SARS). Its outbreak began on mid-December 2019 from Wuhan City, Hubei Province in China, and is subsequently spreading into many countries worldwide at a rapid pace. The recent emergence of the COVID-19 disease underscores limitations in our understanding of various aspects pertaining to the virus, including risk factors that facilitate virus transmission, infection, its development, its severity, its spectrum, and

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their impact on society to guide and inform solutions and countermeasures. Early evidence suggests older age and the presence of underlying comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancer,[1] smoking, obesity,[2] and male gender,[3] predisposes individuals to infection and an increased risk of death. It is now known that the virus inflicts harm irrespective of age or health status. It is therefore important to fully identify genetic, psychological, environmental, and biological risk factors that may influence the course and severity of the disease.[4]

Deficiencies in minerals and vitamins have been reported to be a predisposing factor in a variety of diseases.[5] Vitamin D, in particular, has been studied for decades with a more recent emphasis on its role, in deficient states, in cancers, neuropsychiatric disorders, autoimmune diseases,[5] and chronic conditions such as bone metabolic disorders, cardiovascular disease, chronic respiratory tract infections (RTIs), diabetes mellitus, hypertension and in viral infections.[6] High serum levels of 25-hydroxyvitamin D [25 (OH) D] have been associated with reduced risk of dengue, hepatitis, herpesvirus, hepatitis B and C viruses, human immunodeficiency virus, influenza, respiratory syncytial virus infections, and pneumonia.[6] The exact mechanisms of the interplay between Vitamin D and viral disease development are not fully poorly understood but are thought to involve the induction of an anti-viral state, functional immunoregulatory features, interaction with cellular and viral factors, induction of autophagy and apoptosis, and genetic and epigenetic alterations.[7] Now, with the emergence and rapid spread of COVID-19, the influence of Vitamin D status in infected individuals on the clinical outcomes is an area of interest. COVID-19 incidence has first ranked between others in Iran as an Eastern Mediterranean Region countries.[8] The incidence rate, severity, and death risk of influenza, pneumonia, and the current COVID-19 may reduce by optimal levels of 25-hydroxyvitamin D.[6] As Vitamin D suboptimal levels in Iranian general population was reported in over 70%.[9] The present study was aimed to evaluate the COVID-19 suffered patients 25(OH)D serum concentration which is not reported yet.

EXPERIMENTAL SECTION

Study design, setting, and participants

This cross-sectional study was performed in Firoozgar Hospital, Tehran, an affiliate to Iran University of Medical Sciences, Tehran in Iran. The hospital served as a referral center of COVID-19 patients. All patients admitted to the hospital, from January 16 to March 21, 2020, were enrolled in the study by expert specialists using the standard protocol of WHO interim guidance for COVID-19 diagnosis. Informed consent was obtained from patients who, if in agreement to participate, signed consent form.

Inclusion criteria were the presence of signs and symptoms of respiratory infection in addition to at least a chest computed tomographic (CT) scan or X-ray and an RT-PCR positive for COVID-19. Patients who had not undergone a Vitamin D assessment were excluded from the study.

The medical records of recruited patients were collated by the research team of the Department of Gastrointestinal and Liver Disease Research Center at the Iran University of Medical Sciences. Some demographic, clinical, and laboratory data of recruited patients were reviewed to find the eligible ones (not shown). We included some clinical and laboratory data [Table 1]. The clinical outcomes (i.e., length of hospital stay, discharges, and mortality) were monitored up to the end date of the study, March 21, 2020. The study ethics approval was obtained from Iran University of Medical Sciences, Tehran, Iran (code IR.IUMS.REC.1399.012).

Data collection and measurements

Venous blood samples were obtained from each participant at the time of the Hospital admission. Serum separator tube used for blood clotting and sera obtained after centrifugation. An Auto-Analyzer BS200 (Mindray, Shenzhen, China) used for serum 25-hydroxycholecalciferol (25-OH Vitamin D) assessment by commercial diagnostic kits from Pars Azmoon Company (Pars Azmoon Co., Tehran, Iran). The serum 25(OH)D concentration cut-off clustered based on values >30 ng/ml considered as sufficient, 21–29 ng/ml as insufficient, and ≤20 ng/ml as deficient.

COVID-19 one-step Reverse Transcription Real-time Polymerase Chain Reaction (RT-PCR) (CAPITAL™ qRT-PCR Probe Mix, biotechrabbit, GmbH, Germany)

Table 1: Characteristics of patients with coronavirus disease 2019 infection in the present study

Variable	Categories	Percent (*n*)
Sex	Male	62.5 (198)
	Female	37.5 (119)
Age group	18-39	9.5 (30)
	40-64	43.5 (138)
	≥65	47.0 (149)
ICU admission	Yes	12.0 (38)
	No	88.0 (279)
Hospital length of stay (days)	≤3	51.7 (164)
	4-7	32.5 (103)
	>7	15.8 (50)
Outcome	Survivors	86.8 (275)
	Nonsurvivors	13.2 (42)
Vitamin D (ng/ml)	≥30 (normal)	24.3 (77)
	20-29 (insufficiency)	27.1 (86)
	<20 (deficiency)	48.6 (154)

ICU=Intensive care unit

was performed by a referral diagnostic laboratory which was approved by Iran University of Medical Sciences, Tehran, Iran. Nasopharyngeal swab samples, collected from suspected SAR-Cov-2 patients, were placed into tubes containing of 0.5 ml viral transport media and used for commercial RNA extraction within 24 h. Chest CT images were categorized as abnormal, bilateral, or single-lung involvement by sophisticated radiologists.

Statistical analysis

ALL statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 22 software (SPSS Inc., Chicago, IL, USA). Category variables were calculated as frequencies and percentages. The continuous variables are described by mean, median, and ranges. The Student's *t*-test and Mann–Whitney test were used to compare the continuous variables when data were normally distributed. The WALD Chi-square and Fisher's exact test were used for analysis of correlation between different qualitative variables. Multiple logistic regression was performed to determine the association between mortality and Vitamin D levels and other potential covariates. The $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The study population involved a total of 317 hospitalized COVID-19 positive patients. Of these 12% (38) of patients were admitted and transferred to the intensive care unit (ICU). Males comprised 62.5% (198) of the study cohort while females numbered 119. The total mean age ± standard deviation of patients was 62.34 ± 16.8 years (range: 18–101 years) with 9.5% of patients in the 18–40 year age group, 43.5% in 40–64 years and 47% were ≥65 years [Table 1]. The mean Vitamin D level was 21.98 ± 13.17. The frequency of death and Vitamin D deficiency was 13.2% and 48.6%, respectively [Table 1].

The mean age of survivors and nonsurvivors was 60.49 ± 15.63 years and 72.12 ± 12.87 years respectively ($P < 0.001$). The mean length of stay in hospital for survivors and nonsurvivors was 3.83 ± 3.39 days, and 3.69 ± 3.06 days respectively ($P < 0.001$).

Table 2 shows the frequency distribution of sex, age group, ICU admission status, and hospital length of stay in patients with COVID-19, according to mortality outcome A significant association was observed between mortality outcome and patient age groups ($P = 0.002$) as well as ICU admission ($P < 0.001$).

Tables 3 and 4 show the mean and distribution frequency of Vitamin D level based on ICU admission status, hospital length of stay, and death. Based on our results, the vitamin level in nonsurvivors was significantly lower than survivors ($P = 0.005$) more details are presented in Tables 3 and 4.

Table 5 shows the results of multiple logistic regression analysis in which death was considered the related outcome and sex, age, length of stay, ICU admission, and Vitamin D serum level were considered the potential predictors. Age, admission in ICU, and Vitamin D serum level showed a significant association with mortality. A higher level of serum Vitamin D showed significant protection in survival outcome (odds ratio = 0.955 [95% confidence interval = 0.923–0.988)]).

DISCUSSION

We evaluated the association between Vitamin D levels in patients with COVID-19 disease and related outcomes including, the need for hospitalization in ICU and mortality rate. Our study showed a significant independent association between Vitamin D level and mortality rate in these groups of patients, in that, a lower serum Vitamin D was linked to a higher mortality rate in patients with COVID-19. There was a significant association between Vitamin D level and the ICU admission.

We know the most important cause of death in people with COVID-19 is respiratory involvement.[6,7] On the other hand, the previous studies have reported on an association between respiratory diseases[10-12] and a lower level of serum Vitamin D Chen *et al*. proposed several mechanisms at the molecular level[10] in the relationship between Vitamin D and pathological respiratory distress. Jolliffe *et al*. reported a broad and consistent relationship between lower levels of Vitamin D and a higher susceptibility to acute respiratory infection in a systemic review, although, the authors were unable to conclude that Vitamin D supplementation has a protective effect against acute respiratory infection.[11] However, Martineau *et al*. concluded that Vitamin D supplementation was safe and effective against acute RTI, particularly in patients with severe Vitamin D deficiency.[12] In another systematic review and meta-analysis, Pham *et al*. showed that Vitamin D plays a critical role against acute RTI risk and its severity, particularly in individuals with Vitamin D deficiency.[13] It is worth noting that previous studies proposed that the Vitamin D has an important immunomodulatory role in the optimal function of the immune system.[8,9] The steroid hormone may act against related pathogens and reduce the inflammatory responses.[14-16] Vitamin D has a role in the production of some antimicrobial peptides such as defensins and cathelicidin and thus can play a role against microbiological pathogens.[17]

Table 2: Frequency distribution of mortality outcome relative to gender and age groups of patients with coronavirus disease 2019

Variable	Categories	Mortality outcome (%)		*P*
		Nonsurvivor (*n*=42)	Survivor (*n*=275)	
Sex	Male (*n*=198)	71.4	61.1	0.198
	Female (*n*=119)	28.6	38.9	
Age group	18-39	2.4	10.5	0.002
	40-64	23.8	46.5	
	≥65	73.8	42.9	
ICU admission	Yes	45.2	6.9	<0.001
	No	54.8	93.1	
Hospital length of stay (days)	≤3	64.3	49.8	0.217
	4-7	23.8	33.8	
	>7	11.9	16.4	

A *P*<0.05 denotes statistical significance. ICU=Intensive care unit

Table 3: Mean and distribution frequency of Vitamin D level based on intensive care unit admission status, hospital length of stay and death

Variable	Categories	Mean±SD	*P*
ICU admission	Yes	22.14±12.92	0.575
	No	20.86±15.01	
Hospital length of stay (days)	≤3	22.63±13.83	0.650
	4-7	21.47±12.66	
	>7	20.93±12.05	
Death	Yes	16.74±9.83	0.005
	No	22.78±13.44	

SD=Standard deviation; ICU=Intensive care unit

Table 4: Outcome of Vitamin D levels based on three categories of deficiency, insufficiency and normal

Outcome	Outcome status	Vitamin D status	% (*n*)	*P* value
ICU admission	Yes (*n*=38)	Normal	26.3 (10)	0.236
		Insufficiency	15.8 (6)	
		Deficiency	57.9 (22)	
	No (*n*=279)	Normal	47.3 (132)	
		Insufficiency	28.7 (80)	
		Deficiency	24.0 (67)	
Length of stay (days)	≤3 (*n*=164)	Normal	26.8 (44)	0.689
		Insufficiency	26.2 (43)	
		Deficiency	47.0 (77)	
	4-7 (*n*=103)	Normal	19.4 (20)	
		Insufficiency	30.1 (31)	
		Deficiency	50.5 (52)	
	>7 (*n*=50)	Normal	26.0 (13)	
		Insufficiency	24.0 (12)	
		Deficiency	50.0 (25)	
Death	Yes (*n*=42)	Normal	11.9 (5)	0.089
		Insufficiency	26.2 (11)	
		Deficiency	61.9 (26)	
	No (*n*=275)	Normal	26.2 (72)	
		Insufficiency	27.3 (75)	
		Deficiency	46.5 (128)	

Deficiency=<20 ng/ml; Insufficiency=20-29 ng/ml; Level normal=≥30 ng/ml. ICU=Intensive care unit

Recent studies show that the mortality rate in people with COVID-19 occurs at a higher rate in older patients.[1] Kim *et al*. demonstrated an association of low serum level of Vitamin D with frailty in elderly people.[18] On the other hand, frailty was considered as a strong predictor of mortality rate in older patients.[19] This association was independent of gender, age, other morbidities, and even functional status of involved older adults.[19] As a result, frailty can be considered as an important mediator between lower levels of Vitamin D and higher mortality rate in older patients. On the other hand, the recent studies showed the cardiovascular comorbidities are common findings in patients with COVID-19, where myocardial injury occurs in more than 25% of acute ill patients, and also it is related to a higher risk of death in involved patients.[20] This injury may occur in two patterns, including acute myocardial injury and injury due to illness intensification.[20]

Our study showed that a lower level of serum level of Vitamin D is associated with a higher mortality rate in patients with COVID-19 independent of age and sex. This preliminary study, to the best knowledge of the authors, is the first to evaluate the association between serum level of Vitamin D and mortality rate in patients with COVID-19 disease. We suggest that the role of Vitamin D in SARS-Cov-2 infection be fully explored in infection and at all stages of disease in well-designed clinical studies.

Our study had some limitations. We evaluated the association between serum baseline Vitamin D level and mortality rate in an observational study. Consequently, we were not able to determine whether Vitamin D deficiency could be considered a factor in increasing patient mortality or whether the severity of the disease had significantly reduced Vitamin D levels in those who succumbed. Although we adjusted the related results based on age and sex, some other confounding variables such as underlying diseases were not included. However, the inclusion of ICU admission and hospital length of stay in logistic regression models can help moderate some of the potentially influential variables on the need for hospitalization that are also

Table 5: Multiple logistic regression analysis of different variables by death outcome

Variable	Wald χ^2	OR (95% CI)	*P*
Sex	1.430	1.673 (0.720-3.888)	0.232
Age	11.090	1.049 (1.020-1.078)	0.001
Length of stay	0.007	1.005 (0.900-1.122)	0.934
ICU admission	25.525	8.567 (3.723-19.710)	<0.001
Vitamin D	7.135	0.955 (0.923-0.988)	0.008

A *P*<0.05 denotes statistical significance. CI=Confidence interval; ICU=Intensive care unit; OR=Odds ratio

associated with death. Further research is proposed to fully elucidate the role for Vitamin D in the prevention and treatment of patients with COVID-19. In addition, due to the lack of previous studies in this area, we were unable to adequately evaluate the findings with similar studies.

CONCLUSION

Lower Vitamin D level was significantly associated with the mortality rate of patients confirmed with COVID-19, independent of age and sex.

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Conflicts of interest

There are no conflicts of interest.

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