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Vitamin D assessment in patients with COVID-19 virus and correlation with severity

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Abstract

Background: Vitamin D may play a vital role in preventing the multi-system consequences of COVID-19 infections. The aim of this study is to evaluate the potential association between mean serum levels of vitamin D and COVID-19 and its correlation with severity and mortality.

Results: A case-control study conducted on 80 Egyptian patients admitted at Ain Shams University designated hospitals, Cairo, Egypt, from March 2021 to September 2021. Regarding the laboratory investigations, we found that COVID-19 cases have significantly lower lymphocytic counts than controls. Regarding vitamin D, this study showed a statistically significant positive correlation between vitamin D and lymphocytes, and there were statistically significant negative correlations between vitamin D, neutrophil-lymphocyte ratio, C-reactive protein, blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, ferritin, lactate dehydrogenase, and D-dimer.

Conclusion: This study confirms that vitamin D deficiency is associated with the severity of COVID-19 clinically and laboratory.

Keywords: Vitamin D, COVID-19 virus, Neutrophil-lymphocyte ratio

Background

The role of vitamin D in COVID-19 is not well known since the virus pathophysiology is still new to us. But there is a reason sounding behind this speculation [1].

T regulatory lymphocytes (TRegs) are the main defense mechanism against uncontrolled inflammation. The number of TRegs was found to be markedly lower in patients with severe COVID-19 infection while increased levels were associated with a reduced level of severe respiratory disease. TReg levels can be increased by vitamin D intake. Also, low serum vitamin D concentrations are usually associated with a rise in inflammatory cytokines which raises the possibility that normal serum vitamin

D concentration may reduce the incidence of cytokine storm which can occur in severe COVID-19 infection [2].

Vitamin D deficiency is an important risk factor for infections due to insufficient innate immune system reactivity, so, testing of serum vitamin D at the wintertime (as it has usually the lowest levels expected) should be mandatory to be done as part of regular health check-up status. The test is more vital especially in patients with chronic illnesses, where we should expect vitamin D insufficiency throughout the year with an increased burden of disease severity up to inevitable mortality [2].

Also, thrombotic complications especially in the pulmonary microvasculature are common in COVID-19 and vitamin D deficiency is also associated with a rise in thrombotic events which increases further speculation about the significance of adequate serum vitamin D concentration in COVID-19 [2].

The aim of this study is to assess if there is a potential association between mean serum levels of vitamin D

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and COVID-19 and its correlation with the severity of COVID-19.

Methods

This was a case-control study conducted at Ain Shams University designated hospitals from March 2021 to September 2021. The study population included 80 adult participants ≥ 18 years. All patients underwent a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay for COVID-19 for nasopharyngeal and oropharyngeal swab specimens.

An informed written consent was obtained from all patients before inclusion in this study. The study was approved by the Ethical Review Board of our institution (M S 742 /2020/ 2021). The study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

The participants were divided into two groups: Group I (control): 20 apparently healthy subjects who were previously on vitamin D supplementation and stopped treatment 3 months before enrollment in the study. Group II (patients): 60 COVID-19-positive patients that were further divided into three groups according to the severity of the disease. Group IIA: 20 mild symptomatic COVID-19-positive patients. Group IIB: 20 moderate to severe symptomatic COVID-19 positive patients. Group IIC: 20 critically ill COVID-19-positive patients.

COVID-19 severity was classified as mild, moderate, severe, or critical according to World Health Organization (WHO) guidelines. Mild cases are symptomatic patients without evidence of viral pneumonia or hypoxia. Moderate cases are patients with clinical signs of pneumonia and pneumonic manifestations that can be seen in chest imaging but no signs of severe pneumonia including SpO₂ $\geq 90\%$ on room air. Severe cases include patients with clinical signs of pneumonia and one of the following: respiratory rate >30 breaths/minute, severe

respiratory distress, or SpO₂ $<90\%$ on room air. Critical cases are patients meeting any of the following criteria: occurrence of respiratory failure requiring mechanical ventilation, presence of septic shock, and multi-organ failure that requires monitoring and treatment in the intensive care unit (ICU).

Exclusion criteria

Exclusion criteria include patients with dementia, patients with learning disability, patients with mental health needs and alcohol or drug dependency, pregnant women, and patients with sarcoidosis, hypercalcemia, and known vitamin D intolerance.

All patients were subjected to the following: full history taking, full clinical examination, laboratory investigations that included: PCR for oropharyngeal and nasopharyngeal swab specimens, complete blood count [white blood cell count (WBC), lymphocytic count, hemoglobin and platelet count], neutrophil-lymphocyte ratio (NLR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), kidney function tests [serum creatinine, blood urea nitrogen (BUN), and uric acid], liver function tests and enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total/direct bilirubin and serum albumin], ferritin, lactate dehydrogenase (LDH), D-Dimer, and Vit D (25 hydroxy cholecalciferol) by ELISA. High resolution computed tomography (HRCT) chest was done to determine the COVID-19 Reporting and Data System (CORADs).

Statistical analysis

Data were collected, revised, coded, and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as numbers and percentages while quantitative data were presented as

Table 1 Demographic data of patient groups with mild, moderate, and severe COVID-19 manifestations and control subjects

	Mild n=20	Moderate n=20	Severe n=20	Control n=20	P value
Age (years) (Mean \pmSD)	55.400 \pm 16.791	59.550 \pm 13.953	62.750 \pm 15.003	25.700 \pm 3.197	<0.001
Sex (n, %)					
Male	13 (65%)	9 (45%)	13 (65%)	11 (55%)	0.522
Female	7 (35%)	11 (55%)	7 (35%)	9 (45%)	
Smoking (n, %)					
Smokers	13 (65%)	16 (80%)	10 (50%)	18 (90%)	0.030
Non-smokers	7 (35%)	4 (20%)	10 (50%)	2 (10%)	
Medical history (n, %)					
Free	9 (45%)	3 (15%)	5 (25%)	20 (100%)	<0.001
HTN	6 (30%)	6 (30%)	6 (30%)	0	
DM	2 (10%)	5 (25%)	0	0	
AF+HTN	0	2 (10%)	1 (5%)	0	
DM+HTN	3 (15%)	4 (20%)	8 (40%)	0	

P value >0.05 : non-significant (NS), P value <0.05 : significant (S), P value <0.01 : highly significant (HS)

Table 2 Laboratory and radiologic findings of COVID-19 patient groups: mild, moderate, and severe and control subjects

	Mild n=20	Moderate n=20	Severe n=20	Control n=20	P value
TLC (10⁹/L) (Mean ±SD) (range)	5.24±2.029 2.3–10.2	12.7±6.56 4.3–32.5	12.3±7.8 3.3–33	7.24±1.84 4.5–10.2	<0.001
Lymphocytes (10⁹/L) (Mean ±SD) (range)	0.85±0.344 0.5–2	0.77±.533 0.3–2	0.48±0.337 0.2–1.4	2.010±0.799 1–3	<0.001
Neutrophils (10⁹/L) (Mean ±SD) (range)	4.07±2.05 1–9	11.085±6.337 3.7–30.7	10.87±7.48 2.2–31.9	4.830±1.913 2–8	<0.001
NLR (Mean ±SD) (range)	5.25±3.127 1–13	20.6±13.59 2–43	31.90±26.80 2–106	2.8±1.47 1–6	<0.001
Hemoglobin (g/dl) (Mean ±SD) (range)	12.9 ±2.14 10–18.5	11.78 ±2.555 6.5–15	11.96 ±2.491 7–18	12.185 ±1.267 10–14.5	0.385
Platelet (10⁹/L) (Mean ±SD) (range)	5.25 ±3.127 1–13	20.65 ±13.59 2–43	31.9 ±26.8 2–106	2.8 ±1.47 1–6	<0.001
CRP (mg/dl) (Mean ±SD) (range)	24.5 ±19.8 0.1–62	30.95 ±26.95 11.28–100	78.99 ±68.10 10.7–230	4.15 ±2.09 0.5–6	<0.001
ESR (mm/hr) (Mean ±SD) (range)	24.8 ±17.8 5.1–70	26.55 ±23.23 10–80	44.01 ±28.17 10–90	3.67 ±2.34 0.5–6	<0.001
BUN (mg/dl) (Mean ±SD) (range)	14.8 ±5.6 8–29	41.1 ±21.59 15–88	53.45 ±28.34 17–105	10.0 ±3.62 5–17	<0.001
S.creatinine (mg/dl) (Mean ±SD) (range)	0.86 ±0.34 0.3–1.9	1.03 ±0.41 0.4–2.1	1.5 ±0.81 0.5–3.1	0.82 ±0.30 0.4–1.5	<0.001
AST (IU/L) (Mean ±SD) (range)	24.6 ±6.41 15–42	53.05 ±47.5 9–215	72.0 ±58.44 4–212	14.85 ±6.029 5–25	<0.001
ALT (IU/L) (Mean ±SD) (range)	31.6 ±13.9 11–69	37.9 ±30.19 9–129	49.75 ±38.09 13–138	14.9 ±7.33 7–40	0.001
Total bilirubin (mg/dl) (Mean ±SD) (range)	0.87 ±0.29 0.4–1.5	1.02 ±0.265 0.6–1.6	0.84 ±0.35 0.4–1.6	0.69 ±0.22 0.3–1	0.006
S.albumin (g/dl) (Mean ±SD) (range)	3.43±0.26 3.1–3.9	3.21±0.59 1.8–4.2	3.02±0.54 2.1–3.8	3.66±0.26 3.5–4.5	<0.001
Ferritin (ng/ml) (Mean ±SD) (range)	245.6±135.5 56–677.2	589.6±368.3 112.5–1434	683.4±556.6 26.7–2100	73.0±48.96 20–210	<0.001
LDH (IU/L) (Mean ±SD) (range)	256.4±73.1 110–350	487.5±303.2 125–963	603±239 225–986	160±19.47 140+200	<0.001
D-dimer (mg/L) (Mean ±SD) (range)	0.45±0.19 0.2–0.9	1.16±0.97 0.212–2.9	2.2±1.755 0.3–6.9	0.309±0.08 0.1–0.45	<0.001
Vitamin D (ng/ml) (Mean ±SD) (range)	24.00±3.7 17–30	21.5±3.95 11–27	16.6±4.16 7–23	56.65±14.9 35–89	<0.001
CORADS (n, %)	1 0	0	0	20 (100%)	<0.001
	3 15 (75%)	10 (50%)	3 (15%)	0	
	4 3 (15%)	6 (30%)	5 (25%)	0	
	5 2 (10%)	4 (20%)	12 (60%)	0	

P value >0.05: non-significant (NS), P value <0.05: significant (S), P value < 0.01: highly significant (HS)

mean, standard deviations, and ranges when their distribution was found parametric.

The comparison between two groups with qualitative data was done by using chi-square test and/or Fisher exact test was used instead of chi-square test when the expected count in any cell was found less than 5.

The comparison between two independent groups with quantitative data and parametric distribution was done by using independent *t*-test.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *p*-value was considered significant as the following: $P > 0.05$ = non-significant (NS), $P < 0.05$ = significant (S), and $P < 0.001$ = highly significant (HS).

Results

Results are shown in Tables 1, 2, 3, and 4.

Receiver operating characteristic (ROC) curve was plotted to identify the best cut-off value of vitamin D to predict COVID-19 cases. As shown in Table 5 and Fig. 1, vitamin D at cut-off value ≤ 30 ng/ml have a sensitivity and specificity of 100%.

Discussion

Vitamin D insufficiency had been found to contribute to acute respiratory distress syndrome, the main cause of death caused by COVID-19. Vitamin D plays a vital role in strengthening the body's immunity by stimulating monocyte differentiation and suppressing lymphocyte proliferation [3].

Table 3 Relation between vitamin D and sex, special habits, medical history, and CORADS classification

		Vitamin D (ng/ml)		T-test or ANOVA	
		N	Mean \pm SD	T or F	P value
Sex	Male	35	20.000 \pm 5.358	-1.295	0.200 δ
	Female	25	21.680 \pm 4.318		
Smoking status	Non-smoker	39	21.667 \pm 4.664	2.107	0.039 δ
	Smoker	21	18.905 \pm 5.166		
Medical history	Free	17	20.176 \pm 4.694	1.794	0.143 \cdot
	HTN	18	19.944 \pm 5.557		
	DM	7	23.571 \pm 2.507		
	AF+HTN	3	15.667 \pm 6.429		
	HTN+DM	15	21.867 \pm 4.612		
CORADS classification	CORADS 3	28	24.607 \pm 2.699	71.693	<0.001 \cdot
	CORADS 4	14	20.429 \pm 1.453		
	CORADS 5	18	14.833 \pm 3.365		

P value >0.05 : non-significant (NS), P value <0.05 : significant (S), P value <0.01 : highly significant (HS)

δ Independent *t*-test

\cdot One-way ANOVA

Table 4 Correlation of vitamin D and age, different laboratory, and radiologic findings of COVID-19 patients

Correlations	Vitamin D	
	R	P value
Age (years)	-0.117	0.374
TLC ($10^9/L$)	-0.194	0.138
Lymphocytes ($10^9/L$)	0.270	0.037*
Neutrophils ($10^9/L$)	-0.182	0.164
Hemoglobin (g/dl)	0.124	0.344
Platelets ($10^9/L$)	-0.044	0.737
Neutrophil-lymphocyte ratio	-0.295	0.022*
CRP (mg/dl)	-0.259	0.046*
ESR (mm/h)	-0.167	0.201
BUN (mg/dl)	-0.315	0.014*
Serum creatinine (mg/dl)	-0.256	0.048*
AST (IU/L)	-0.412	0.001*
ALT (IU/L)	-0.337	0.008*
Total bilirubin (mg/dl)	0.037	0.778
S. albumin (g/dl)	0.248	0.056
Ferritin (ng/ml)	-0.463	<0.001*
LDH (IU/L)	-0.269	0.038*
D-dimer (mg/L)	-0.382	0.003*

* P value <0.05 : significant, P value <0.01 : highly significant

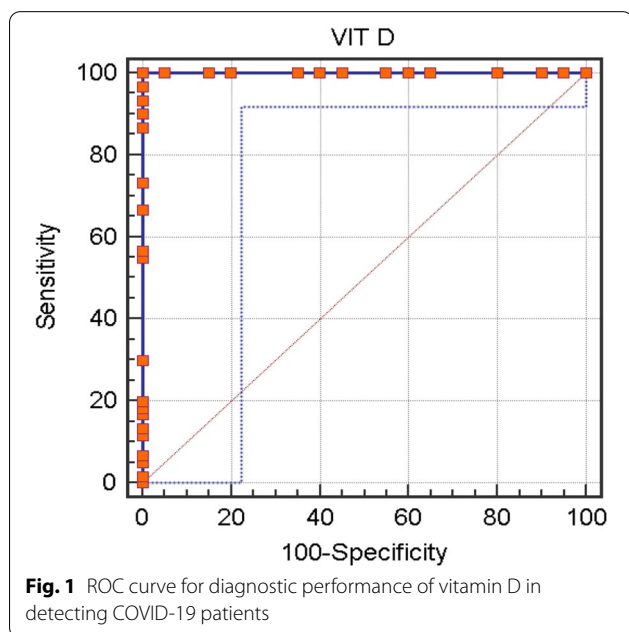
The current study compared vitamin D serum levels with different health-related scores, inflammatory markers, markers of cellular damage and coagulation, and radiological findings during COVID-19 illness. Interestingly, patients with low vitamin D plasma levels had compromised biochemical and clinical findings reflected by the profound immunological involvement. Vitamin D insufficiency and deficiency were common in COVID-19-affected patients.

Regarding the laboratory investigations, [4] found that LDH had increased significantly in most patients, while albumin had decreased, but ALT and AST showed no significant changes. Another study by [5] showed that 2–11% of patients with COVID-19 had liver comorbidities and 14–53% of cases had abnormal ALT and AST levels during the progression of COVID-19 disease which supports our results.

Also, Shi et al. [6] found that incidence of AST abnormality among the patients diagnosed by CT chest was significantly lower than those diagnosed after the onset of symptoms. Therefore, liver injury is more common in severe cases as compared to mild cases of COVID-19. On the other hand, Yang et al. [7] found no significant difference in the liver function between survivors (30%) and non-survivors (28%). Liver injury in mild cases of

Table 5 Diagnostic performance of vitamin D in detecting COVID-19 patients

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Vitamin D	≤30 ng/ml	100%	100%	100%	100%	100%



COVID-19 is usually transient and can return back to normal or its baseline without any special treatment [5].

We had found that the number and percentage of WBC, lymphocytes, and neutrophils were significantly different between positive COVID-19 cases and the control group. In comparison to the normal range, we found low lymphocytic counts in patients with positive COVID-19, whereas neutrophil counts were higher in these patients and this agrees with [8] study.

Viral particles usually spread throughout the respiratory tract and infect other cells stimulating a series of immune responses and causing changes in the white blood cells especially lymphocytes. Some studies suggest that a significant decrease in the number of lymphocytes suggests that the coronavirus affects the immune cells and suppresses the cellular immune function [4]. Tsui et al. [9] reported that increased neutrophil count and elevated LDH levels were independent predictors of an adverse clinical outcome.

Our study showed a statistically significant positive correlation between vitamin D and lymphocytes, and there was a statistically significant negative correlation between vit D, neutrophil-lymphocyte ratio, CRP, BUN, serum creatinine, AST, ALT, ferritin, LDH, and D-dimer.

Meltzer et al. [10] in a retrospective cohort study done at the University of Chicago showed that the rate of COVID-19 was higher in a vitamin D-deficient group than a vitamin D-sufficient group.

Also, [11] in a retrospective cohort study done in Swiss patients showed significantly lower levels of vitamin D in COVID-positive patients (median value 11.1 ng/mL) as compared with negative patients (24.6 ng/mL).

A study done by Kara et al. [12] showed that vitamin D deficiency was a pandemic especially in Europe, where 40% were vitamin D-deficient and 13% severely deficient.

Mendy et al. [13] found that the severity of COVID-19 disease was associated with vitamin D deficiency and the likelihood of ICU admission were higher among vitamin D-deficient patients.

Daneshkhah et al. [14] found that treating severe vitamin D deficiency decreased the risk of increased CRP levels which are used as a marker of cytokine storm, which usually occur in severe COVID-19 cases.

Lau et al. [15] revealed that eleven (84.6%) ICU patients that were respiratory compromised due to COVID-19 and four (57.1%) non-ICU patients had vitamin D deficiency. ICU patients included ten patients with hypertension and six patients with diabetes mellitus. Non-ICU patients included five hypertensive and one diabetic patient. Of these, 64.6% ($n=7$) had low vitamin D levels ($<20 \mu\text{g/mL}$) and 3% had critically low levels ($<10 \mu\text{g/mL}$)

One trial by Grazon [16] showed that intake of a single oral dose of 25,000 IU (625 μg) of vitamin D would improve mortality in patients with COVID-19.

Castillo et al. [17] showed that high doses of calcifediol reduce ICU admission for hospitalized patients.

Conclusion

In conclusion, this study confirms that vitamin D deficiency is associated with the severity of COVID-19 clinically and laboratory.

Acknowledgments

None

Authors' contributions

WM formulated the research idea and shared in data analysis, and manuscript revision. SS supervised data analysis and manuscript drafting. SA shared in data collection, statistical analysis, and manuscript drafting. AA shared in the

formulation of the research hypothesis, data processing, statistical analysis, and drafting of the final manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All subjects involved in the study signed an informed consent to participate. The study was approved by the Ethical Review Board of Ain Shams University (M S 742/2020/2021). The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Consent for publication

Participants provided a consent for the study findings to be published.

Competing interests

All authors declare they have no competing interests.

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References

- Weir EK, Thenappan T, Bhargava M et al (2020) Does vitamin D deficiency increase the severity of COVID-19? *Clin Med (Lond)* 20(4):e107
- Grant WB, Lahore H, McDonnell SL et al (2020) Evidence That Vitamin D Supplementation Could Reduce the Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients* 12(4):988
- Pinzon RT, Angela PAW (2020) Vitamin D deficiency among patients with COVID-19: case series and recent literature review. *Trop Med Health* 48(1):102. <https://doi.org/10.1186/s41182-020-00277-w>
- Chen N, Zhou M, Dong X et al (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395(10223):507
- Zhang C, Shi L, Wang FS (2020) Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 5(5):428
- Shi H, Han X, Jiang N et al (2020) Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis* 20(4):425
- Yang X, Yu Y, Xu J et al (2020) Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 8(5):475
- Qu R, Ling Y, Zhang YHZ et al (2020) Platelet-to-lymphocyte ratio is associated with prognosis in patients with Corona Virus Disease-19. *J Med Virol* 92(9):1533
- Tsui PT, Kwok ML, Yuen H et al (2003) severe acute respiratory syndrome: clinical outcome and prognostic correlates. *Emerg Infect Dis* 9(9):1064
- Melizer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J (2020) Association of vitamin D deficiency and treatment with COVID-19 incidence. 2020.05.08.20095893;465–487
- D'Avolio A, Avataneo V, Manca A et al (2020) 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. *Nutrients* 12(5):1359
- Kara M, Ekiz T, Ricci V et al (2020) 'Scientific strabismus' or two related pandemics: COVID-19 & vitamin D deficiency. *Br J Nutr* 124(7):736
- Mendy A, Apewokin S, Wells AA, Morrow AL (2020) Factors associated with hospitalization and disease severity in a racially and ethnically diverse population of COVID-19 patients. 2020.06.25.20137323;541–548
- Daneshkhan A, Agrawal V, Eshein A, et al (2020) The Possible Role of Vitamin D in Suppressing Cytokine Storm and Associated Mortality in COVID-19 Patients. *medRxiv*. <https://doi.org/10.1101/2020.04.08.20058578>
- Lau FH, Majumder R, Torabi R, Saeg F, Hoffman R, Cirillo JD, Greiffenstein P (2020) Vitamin D insufficiency is prevalent in severe COVID-19. 2020.04.24.20075838;257–281
- Grazon MC. (2020) Vitamin D on prevention and treatment of COVID-19 (COVITD-19). *ClinicalTrials.gov*. <https://clinicaltrials.gov/ct2/show/NCT04334005>.
- Castillo ME, Costa LME, Barrios JMV, Díaz JFA, Miranda JL, Bouillon R, Gomez JMQ (2020) 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. *J Steroid Biochem Mol Biol* 203:105751

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