Assessment of Serum Vitamin D and Total Calcium Levels in COVID-19 Infected Patients and Their Relation to Disease Severity

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Background and study aim: COVID-19 is a highly contagious viral infection that was initially discovered in late 2019 in China. Vitamin D was proposed as a COVID-19 severity indicator by many authors. However, the role of hypocalcemia has not yet been well established. So we evaluated serum levels of vitamin D and total calcium in adult patients infected with COVID-19 and correlated vitamin D and calcium levels with the severity and prognosis of the COVID-19 infection.

Patients and Methods: Our study evaluated 98 patients (50 females, 48 males) who were diagnosed with positive SARS-CoV-2 polymerase chain reaction. The patients were categorized into severe and non-severe COVID-19 based on the Egyptian protocol for the management of COVID-19 patients. Serum vitamin D and calcium levels were estimated in all patients.

Results: In severe COVID-19 individuals, serum vitamin D levels were significantly lower (P = 0.04). However, there was no statistically significant change in serum calcium levels between severe and non-severe COVID-19 patients (P = 0.7) despite the high prevalence of hypocalcemia in our cohort (88%, 86/98). There was no statistically significant difference in the blood levels of both vitamin D and calcium between the improved group of patients and the death group (P = 0.5, 0.1 respectively).

Conclusion: Vitamin D deficiency is a risk factor for disease progression and severity in COVID-19 patients. Hypocalcemia is not linked to COVID-19 severity despite its high prevalence in these patients. However, to back up these findings, more research with bigger sample size is required .

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly infectious disease that was in Wuhan, China, in December 2019. In recent months, the infection has spread throughout many healthcare systems worldwide. It was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) **[1]**. Cough, fatigue, fever, dyspnea, and sore throat are the most frequent clinical presentation of COVID-19 **[2]**. However, the disease spectrum has a wide scale of severity that varies according to the virus variant **[3]**.

Previous studies have suggested many risk factors for severe COVID-19 infection. Old age **[4]** and male sex **[5]** are principal factors in the suggested clinical severity risk scores. Co-morbidities, such as diabetes mellitus **[6]**, chronic kidney disease, chronic lung diseases, hypertension, and immunosuppression worsen the prognosis and increase the chance of intubation and death **[4]**.

As regards, laboratory investigations raised D-dimer levels **[7]**, lymphopenia **[8]**, and raised aminotransferases (AST and ALT) levels **[9]** are considered risk factors for bad prognosis.

Many studies report a link between vitamin D status and COVID-19 infection. Some retrospective studies demonstrate a strong link between Vitamin D insufficiency and the number of COVID-19 cases **[10]**, COVID-19 severity **[11]**, and the number of deaths **[10]**. Moreover, Vitamin D supplementation (in addition to magnesium and vitamin B12) has a protective role against the clinical deterioration of COVID-19 patients **[12]**.

To our knowledge, the role of hypocalcemia in critically ill patients has been well established **[13]**. Moreover, Hypocalcemia was found in hospitalized patients with many viral infections. It has been identified as a prominent feature in hospitalized patients with SARS virus infection **[14]**, and H7N9 influenza virus infection **[15]**. However, the link between hypocalcemia and COVID-19 severity has not yet been conclusively proven.

Taking into account the above-mentioned considerations, our prospective study aimed to assess serum levels of vitamin D and total calcium in adult patients infected with COVID-19 and correlate the vitamin D and calcium levels with the severity and prognosis of COVID-19.

PATIENTS AND METHODS

A prospective, observational study evaluated 98 patients (50 females, 48 males) who were positive for SARS-CoV-2 by polymerase chain reaction test. After the approval of the study protocol, information was gathered from the COVID-19 isolation hospital at Sohag University between June 1 and June 30 of the year 2021. Patients were followed up for at least one month to evaluate the prognosis of the disease.

All patients who were positive for SARS-CoV-2, except pregnant women, patients with chronic renal failure, patients with malabsorption, and organ transplant recipients were included in this study. After obtaining each participant's informed consent, the authors asked about important complaints such as fever, fatigue, cough, dyspnea, and diarrhea. A10 ml of blood was taken and used to measure serum Vitamin D and calcium levels, complete blood count (CBC), AST, ALT, D-dimer, C-reactive protein (CRP), ferritin, creatinine, and random blood sugar. Vitamin D was measured by the immunoassay method using the I Flash 1800 automated chemiluminescence analyzer. The Calcium was measured by the spectrophotometric method using the photometer 5010.

The patients were categorized into severe and non-severe COVID-19 based on the Egyptian protocol for the management of COVID-19 patients. Severe cases presented with a respiratory rate of more than 30 with room oxygen saturation of less than 92 and a CT chest showed 50% lung involvement or progressive lung lesions within 24 to 48hrs[16].

Statistical analysis: The Statistical Package for the Social Science (SPSS) version 16 was used to interoperate the information. The mean, standard deviation, minimum, maximum, median, and interquartile range (IQR) were used to present quantitative data. Frequencies, percentages, and the $\chi 2$ tests were used for qualitative data. For skewed continuous data, the Mann-Whitney test was used to compare mean values between groups, and the Spearman correlation coefficient was used to assess the degree of correlation between quantitative data. The student's t-test was used to compare the means of normally distributed data.

RESULTS:

The study was conducted on 98 patients, comprising 48 (49%) men and 50 (51%) women. These patients' ages ranged from 24 years to 88 years, with a mean value of 59.9 years. Sixty-three had a non-severe disease while 35 patients had severe disease. All severe cases needed Intensive Care Unit (ICU) admission. The predominant co-morbidity in our participants was hypertension which was present in 46 cases (46.9%) followed by diabetes mellitus in 42 cases (42.9%. There were no significant differences in the rates of comorbidities between

patients with severe diseases and those with non-severe. As regards the clinical manifestations fever was the most common presenting symptom. The serum ferritin level was higher in patients with severe COVID-19 than in those with non-severe COVID-19 (0.05). On the other hand, serum vitamin D levels were significantly lower in patients with severe COVID-19 (p-value = 0.04). There was no statistically significant difference in serum calcium levels between patients with severe and non-severe COVID-19 (p-value = 0.7) **(Table 1).** Moreover, when we do univariate and multivariate logistic regression, we found that Vitamin D independent predictor of the severity of COVID19.

Both males and women with COVID-19 had considerably lower serum vitamin D levels than those with non-severe COVID-19 (p-value = 0.02) **(Table 2).**

Table 3 summarizes the clinical, epidemiological, and laboratory characteristics of the study participants concerning their vitamin D and calcium statuses. Eighty-four patients had vitamin D deficiency and 86 had calcium deficiency. Most patients with decreased vitamin D levels and calcium deficiency were females (55% and 56% respectively). Dyspnea was the most reported symptom in both patients with low vitamin D and those with calcium deficiency. Fifty-six percent of patients with vitamin D deficiency and 50% of patients with calcium deficiency were CORAD 5 on CT scans.

The serum levels of vitamin D and calcium had a strong negative correlation with the patients' ages (P = 0.002, 0.007, respectively). The levels of serum vitamin D and serum creatinine had a strong positive correlation (P = 0.03) **(Table 4).**

During follow-up, out of 98 patients, 36 were excluded due to incomplete clinical data, missing blood test results, or irregular follow-up. Seven (11.3%) patients developed diabetes mellitus. COVID-19 was completely resolved in 53 (85.5%) patients. The death occurred in nine (14.5%) patients; however, there was no statistically significant difference in the blood levels of both vitamin D and calcium between the improved group of patients and the dead one **(Table 5).**

Table (1): Clinical, epidemiological, and laboratory characteristics of the studied population.

	Total N (%)	Non-severe COVID-19 N=63	Severe COVID-19 N=35	*P-Value*
Age (year), Median (IQR)	62 (52.5- 68)	62 (53-68)	62 (50-68)	0.9
Gender				
Male	48 (39.8%)	27(42.9%)	14(40%)	0.1
Female	50 (51%)	36(57.1%)	21(60%)	
Co-morbid conditions(n%)				
Diabetes mellitus	42 (42.9%)	24(38.1%)	18(41.5%)	0.2
Hypertension	46 (46.9%)	28(44%)	18 (51%)	0.5
Chronic obstructive lung disease	11(11.5%)	6(5.6%)	5 (14.3%)	0.2
Chronic liver diseases	9 (9.2%)	5 (7.9%)	4 (11.4%)	0.5
Reported Symptoms(n%)				
Fever	85(86.7%)	55(87.3%)	30(85.7%)	0.8
Cough	64 (65.3%)	44(96.8%)	20(57.1%)	0.2
Dyspnea	75(76.5%)	50(79.4%)	25(69.5%)	0.6
Chest pain	13 (13.3%)	8(12.7%)	5(14.5%)	0.8
Diarrhea	9 (9.2%)	7 (11.1%)	2(5.7%)	0.4
Abdominal pain	23 (23.5%)	16(25.4)	7 (20%)	0.5
CT chest findings (n%)				
CORAD 3	10 (10.2%)	9 (14.3%)	1 (2.9%)	0.001`
CORAD 4	37 (37.8%)	30 (47.6%)	7(20%)	
CORAD 5	51 (52%)	24 (38.1%)	27 (77.1%)	
Laboratory investigation, Median (IQR)				
WBCS (109/l)	7.8 (6-12.8)	7.3(5.4-12)	9.9 (6.2-13)	0.3
Lymphocyte (%)	19.3 (9.7- 37.6)	20 (9.9-39.3)	15 (8.2-33.9)	0.5
Hemoglobin (g/dl)	12.7 (11- 13.5)	12..7(11-13.3)	12.6 (11-13.7)	0.7
Platelets(109/l)	229 (181-288)	226 (178-300)	232(197-300)	0.9
ALT (U/L)	35 (24-53.8)	33 (24-52)	39 (26-68)	0.3
AST (U/L)	52 (29.8- 98)	55 (30-98)	50 (29-97)	0.8
CRP (mg/dl)	24 (16.9-48.5)	24 (18.3-48)	24 (14-67)	0.9
Ferritin (ng/ml)	289 (125.8-678.5)	188 (118-528)	545(135-766)	0.05
D-dimer (ng/ml)	600 (400-785)	580 (400-800)	600(430-720)	0.8
Glucose (mg/dl)	165(20-231)	160 (119 -280)	187(123-234)	0.5
Creatinine (mg/dl)	1.1(0.98-1.6)	1.03 (0.9-1.36)	1.2(1-1.6)	0.08
Vitamin D (ng/ml)	17.5 (14.8 – 22.3)	17.9 (15.5-23.4)	16 (14.5-19.9)	0.04
Calcium (mg/dl)	8.1 (7.5-8.4)	8.1 (7.5-8.4)	8.1(7.5-8.8)	0.7

ALT: alanine aminotransferase, AST: aspartate aminotransferase, COVID-19: Coronavirus disease 2019, CORAD: COVID-19 Reporting and Data System, CRP: C-reactive protein, CT: computed tomography, IQR: interquartile range, Vitamin D: vitamin D, WBCS: white blood cells.

Table (2): Serum vitamin D and calcium in patients with severe and non-severe COVID-19 according to sex

Serum values	Gender	Non-severe COVID-19 N=63	Severe COVID-19 N=35	P-value
Vitamin D (ng/ml) mean±SD	Male Female	19.3±5.8 22.8±8.6	16±2.3 18.8±5.1	0.02 0.02
Calcium (mg/dl), mean±SD	Male Female	7.9±0.6 8.1±0.7	7.7±0.9 8.2±0.8	0.4 0.6

COVID-19: Coronavirus disease 2019, SD: standard deviation, Vitamin D: vitamin D.

Table (3): Clinical, epidemiological, and laboratory characteristics of the studied population according to vitamin D and calcium levels.

	Patients with deficient Vitamin D ≤30ng/dl N= 84	Patients with normal Vitamin D 30 - 100 ng/dl N=14	*P-value*	Patients with deficient calcium ≤ 9mg/dl N= 86	Patients with normal calcium (9-11mg-dl) N=12	*P-value*
Age (year), Median (IQR)	53(63-69.5)	55 (50-64)	0.07	50(61.5-68)	65 (56.3-68)	0.3
Gender						
Male	38 (45.2%)	4 (28.6%)	**0 .07**	38 (44.2%)	10 (83.3%)	0.01
Female	46 (54.8%)	10 (71.4%)		48(55.8%)	2 (16.7%)	
Reported Symptoms						
Cough	52 (61.9%)	12 (85.5%)	0.08	53(61.6%)	11(91.7%)	0.04
Dyspnea	64 (76.2%)	11 (78.6%)	0.8	67(77.9%)	8 (66%)	0.7
Chest pain	10 (11.9%)	3 (21.4%)	0.3	11(12.8%)	2 (16.7%)	0.6
CT chest findings						
CORAD 3	7 (8.35%)	3 (21.4%)	0.1	8 (9.3%)	2 (16.7%)	0.3
CORAD 4	30 (35.7%)	7 (50%)		35(40.7%)	2 (16.7%)	
CORAD 5	47 (56%)	4 (28.6%)		53(50.0%)	8 (66.7%)	
Laboratory investigation, Median (IQR)						
WBCS (10^9/l)	7.6 (6-12.3)	10.5 (6.8-19)	0.2	7.6(6-12.1)	12(6.4-15.7)	0.2
Lymphocyte (%)	18.3(10.1-35)	22.5(8.1- 67.5)	0.7	19.8(10-37.8)	15.5(8.1-36)	0.6
Hemoglobin (g/dl)	12.6 (11-13.3)	12.9(11.2-14)	0 .4	12.6(10.8-13.3)	12.9(12-14.3)	0.1
Platelets(10^9/l)	222 (180-286)	200(253-389)	0.2	227(180.7-300)	231(198-244.5)	0 .7
ALT (U/L)	34 (24-52)	38.5 (24.8-66)	0.8	34 (23-53.8)	36 (30.5-62.3)	0.7
AST (U/L)	52 (30-95.5)	66 (19.8-114.7)	0.8	51(30-97.3)	80.5 (27-107)	0.6
CRP (mg/dl)	24 (16-48)	23.5(19.8-61.8)	0.7	24(16-48)	48 (20.8-71)	0.1
Ferritin (ng/ml)	305.9(130-681)	147.5(98.4-622)	0.2	256(130-559.7)	380.9(94-714)	0.9
D-dimer (ng/ml)	590(400-700)	710(482.5-905)	0.2	600(400-785)	600(447.5-750)	0.1
Glucose (mg/dl)	161(120-230)	178.5(110.5-264.7)	0.6	161(120-231)	186(129.7-237.5)	0.7
Creatinine (mg/dl)	1.1(0.98-1.6)	1(0.92-1.1)	0 .4	1.1(0.98-1.6)	1.2(0.98-1.7)	0 .5
Vitamin D (ng/ml)	17.2 (14.5-20)	30.3 (30-35.1)	0.000	17.5(15.2-22.1)	20.3(14.2-35.7)	0 .02
Calcium (mg/dl)	8 (7.5-8.4)	8.5 (8.1-9)	0 .007	7.9 (7.5-8.3)	9.2 (9.1-9.5)	0.000

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CORAD: COVID-19 Reporting and Data System, CRP: C-reactive protein, CT: computed tomography, IQR: interquartile range, Vitamin D: vitamin D, WBCS: white blood cells

Table (4): Correlations between vitamin D level and serum calcium level, and age, and laboratory investigation.

Investigation	Vitamin D		Serum calcium	
	correlation co-efficient (r)	P-value	Correlation co-efficient (r)	P-value
Age	-0.98	0.002	-.95	0.007
WBCS	0.2	0.06	0.09	0.3
Hemoglobin	0.09	0.3	0.07	0.5
Platelets	-0.97	0.4	0.08	0.4
ALT	0.08	0.4	0.02	0.9
AST	-0.13	0.2	-.03	0.8
Creatinine	0.2	0.03	0.04	0.7
CRP	0.1	0.2	0.1	0.2
D-dimer	0.09	0.4	0.005	0.9
Ferritin	-0.04	0.7	-.06	0.5
Calcium	0.3	0.003	----------	-------
Vitamin D	----------	----------	0.3	0.003

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, Vitamin D: vitamin D, WBCS: white blood cells

Table (5): Serum vitamin D and calcium levels in COVID-19 patients after a follow-up

	Improved patients N=53	Dead patients N=9	P-value
Vitamin D (ng/ml) Median (IQR)	17.9(15.5-22.95)	20.3(16.4-30.15)	0.5
Calcium (mg/dl) Median (IQR)	8.1 (7.5-8.45)	8.5(8-8.95)	0.1

IQR: interquartile range, Vitamin D: vitamin D.

DISCUSSION

Despite the paucity of conclusive evidence that vitamin D supplementation improves COVID-19 disease outcomes, some researchers investigated the relationship between vitamin D status and COVID-19 incidence **[17]**, and disease severity **[11, 18]**, and the number of deaths **[10, 19]**.

The main outcome of this prospective observational study is that low levels of serum vitamin D are related to severe COVID-19 infection in both males and females.

This outcome might be explained by the importance of the active metabolite of vitamin D [1,25(OH)2-D] in the modulation of the growth and differentiation of different immune cell types **[20]**.

The effect of vitamin D deficiency on bacterial and viral infections has previously been studied thoroughly. Some studies showed that people with vitamin D deficiency are more susceptible to acute respiratory tract infections with a higher risk of a prolonged course **[19]**. Moreover, Vitamin D intake may decrease the rate of pneumonia and bronchitis **[21]**.

Other studies found an association between high seasonal influenza incidence and low vitamin D levels **[22]**. The risk of tuberculosis was found to be twice as high in patients with low serum levels of vitamin D than in those with normal levels **[23]**.

The main outcome of our study is consistent with the observations of many authors who studied the association between vitamin D status and COVID-19 disease severity and prognosis. A retrospective study carried out in China demonstrated that serum levels of vitamin D were significantly lower in patients with severe COVID-19 than in those with non-severe disease **[24]**. Moreover, Sulli et al. reported significantly lower serum vitamin D levels in COVID-19 patients compared to controls. They also reported negative correlations between serum levels of vitamin D and D-dimer, CRP, and the severity of CT findings. Moreover, they reported statistically significant positive correlations between serum levels of vitamin D and the

partial pressure of oxygen (PaO2), oxygen saturation, and the ratio of PaO2 to fractional inspired oxygen. Finally, they discovered that elderly patients who died during hospitalization had considerably lower vitamin D serum levels than survivors **[25]**.

Panagiotou et al. found that vitamin D deficiency was one of the main risk factors for severe acute respiratory distress syndrome in COVID-19 patients who were admitted to the hospital. They also found that vitamin D deficiency was more common in patients requiring ICU admission than in those who did not. Panagiotou et al. found that vitamin D deficiency was among the leading risk factors for severe acute respiratory distress syndrome in hospitalized COVID-19. They also found that decreased vitamin D was more frequent in patients requiring ICU admission than in those who did not **[26]**.

Another retrospective study carried out in Italy found that COVID-19 patients admitted to the Respiratory Intermediate Care Unit due to respiratory failure had a significant prevalence of vitamin D insufficiency. They also observed a higher risk of death among patients with severe vitamin D deficiency (<10 ng/mL) in comparison to others **[27]**.

Moreover, a prospective study carried out by Radujkovic et al. revealed that COVID-19 patients with vitamin D insufficiency required greater oxygen therapy and invasive mechanical ventilation, resulting in a higher hospitalization rate. They also reported that Vitamin D insufficiency was also linked to a 6-fold increased chance of a severe course of infection and a 15-fold increased risk of fatality **[28]**.

The protective effect of vitamin D in COVID-19 patients could be explained by various mechanisms, one of which involves cathelicidin (LL-37), a bactericidal peptide with antiviral activity. It is produced by macrophages and epithelial cells and induced by vitamin D via intracellular receptors [29]. The main mechanism of the hyper-inflammatory lung damage in severe COVID-19 infection is an increase in angiotensin-2 activity. SARS-CoV-2 interacts with angiotensin-converting enzyme 2 receptors to cause this response, which is aggravated by vitamin D deficiency **[30]**. Vitamin D also inhibits the release of pro-inflammatory mediators such as interleukin-17 (IL-17) and interferon-gamma by CD4+ T cells and boosts the production of the anti-inflammatory cytokine IL-10. These effects are significantly stronger in females than in males **[31]**.

According to the results of our study, the effect of serum vitamin D levels on COVID-19-related mortality was not significant. This is in line with Luo et al. [24] findings. The limited number of patients that died could clarify this finding.

Although many authors described a distinct link between hypocalcemia and severe illness and mortality **[13, 32]**, the impact of hypocalcemia on COVID-19 is not well recognized. Hypocalcemia is common in critically ill patients. This could be attributed to many factors such as the increase in parathormone secretion, vitamin D deficiency, poor feeding, hypoproteinemia, hypomagnesemia, and drug interactions **[33]**.

The prevalence of hypocalcemia in our cohort was 88% (86/98), and the median serum calcium level was 8.1 mg/dl among patients with severe and non-severe diseases. Based on the definition of hypocalcemia in our study (serum total calcium <9 mg/dl), the condition was prevalent in both groups. Pal et al. also reported a high prevalence of hypocalcemia among patients with both mild and moderate COVID-19 **[34]**.

The calcium ion is a widely used signaling messenger in all physiological activities of the cell. It is included in all steps of the life cycle of the virus including the formation of virion structure, the passage of the virus into the cell, the expression of a viral gene, the processing of viral proteins, and the maturation and release of the virion **[35]**. Thus, the high prevalence of hypocalcemia among COVID-19 patients could be explained by the disruption in calcium homeostasis by a high viral load **[36]**.

Many publications have reported the crucial role of hypocalcemia in COVID-19 disease progression and severity. Wu et al. identified hypocalcemia as an independent risk factor for hospitalization **[37]**. Sun et al. found that hypocalcemia (≤2.0 mmol/L) was associated with a higher 28-day mortality rate and a higher incidence of organ injury **[38]**. Moreover, Osman et al. found an association between hypocalcemia and ICU admission **[39]**.

The current study has some limitations. First, it is a single-center study with small sample size. Second, we considered total calcium levels without considering ionized calcium levels, which may be misleading as in the case of

hypoalbuminemia **[40]**. Third, most patients were elderly (60–80 years) and had co-morbidities, which may have affected disease outcomes. Moreover, leukocytosis was found in some patients; so, the possibility of bacterial co-infection could not be excluded in these patients. Finally, since some patients were lost to follow-up, the associations between vitamin D deficiency, hypocalcemia, and disease outcomes may be imprecise. As a result, we believe that the findings of our study should be further investigated via larger multicenter studies.

CONCLUSION

Our findings support the theory that vitamin D deficiency is a potential risk factor for disease progression and severity in patients with COVID-19. On the other hand, hypocalcemia is not associated with COVID-19 severity despite its high prevalence in these patients.

Ethical Consideration: The study was done according to the declaration of Helsinki and the sound practices. Informed consent was obtained from all participants in the study. The Ethical Committee of Scientific Research, Faculty of Medicine, Sohag University approved the study procedure with a registration number: Soh-Med-21-06-30.

ClinicalTrials.gov Identifier: NCT 04949412.

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Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase, CBC: complete blood count, COVID-19: Coronavirus disease 2019, CORAD: COVID-19 Reporting and Data System, CRP: C-reactive protein, CT: computed tomography, ICU: Internal Care Unit, IL: interleukin, IQR: interquartile range, PaO2: partial pressure of oxygen, PCR: polymerase chain reaction, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SD: standard deviation, SPSS: Statistical Package for the Social Science, Vitamin D: vitamin D, WBCs: white blood cells.

HIGHLIGHTS

- Vitamin D was proposed as a predictor of COVID-19 severity by many authors. However, the role of hypocalcemia is not well established.
- Serum Vit D level was significantly lower in severe COVID-19 patients (P= 0.04).
- Despite the high prevalence of hypocalcemia in our cohort (88% (86/98), there was no statistically significant difference in serum calcium level between severe and non-severe COVID-19 patients (P= 0.7).
- There was no significant difference between the improved group of patients and the death group as regards the blood levels of both Vit D and calcium.

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