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A Study on Serum Vitamin D3 Level in Patients with Covid-19: A Cross-Sectional Study in Kolkata

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ABSTRACT

Background: Coronavirus disease (COVID-19) is a global pandemic caused by SARS-CoV-2. Vitamin D has immunomodulatory and anti-inflammatory properties, potentially influencing the disease course. This study assessed the prevalence of 25(OH) vitamin D deficiency in COVID-19 patients and its association with disease severity in the Indian population.

Methodology: A hospital-based cross-sectional study was conducted at Medical College, Kolkata, including 100 RT-PCR-confirmed moderate and severe COVID-19 patients. Disease severity was categorized based on oxygen saturation. Serum 25-Hydroxy vitamin D levels were measured on admission, along with other hematological and biochemical parameters. High-resolution CT scans were performed to assess pulmonary involvement.

Results: Vitamin D insufficiency and deficiency were observed in 18% and 67% of patients, respectively. Deficiency was more prevalent in severe cases (82.97%) than moderate cases (52.83%). The mean vitamin D levels in moderate and severe disease groups were 23.23±8.74 and 17.17±8.09 ng/ml, respectively. A significant association was found between vitamin D deficiency and COVID-19 severity (P = 0.006). The vitamin D cutoff for predicting severe disease was 18.57 ng/dl.

Conclusion: Vitamin D deficiency is strongly associated with severe COVID-19 in the Indian population. Low vitamin D levels may predict disease severity, suggesting supplementation as a potential preventive strategy.

Key-words: Coronavirus disease, COVID-19, Vitamin D, 25 Hydroxy Vitamin D, Deficiency

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or COVID-19, was responsible for a global pandemic following its emergence in an outbreak in Wuhan, China. It is caused by a viral RNA genome encoded enveloped, single stranded RNA virus. It was designated as a Public Health Emergency of International Concern by the WHO in the month of January, 2020 and subsequently as a pandemic two months later. Patients with COVID-19 infection may present with a wide range of symptoms, including life-threatening ones alongside a large proportion of asymptomatic carriers. Most patients have symptoms such as fever (83%), cough (82%) and respiratory distress (31%). SARS-CoV-2 uses Angiotensin Converting Enzyme (ACE-2) as an entry receptor to infect respiratory epithelial cells via a process that involves being endocytosed via the said receptors.[1] Severe COVID-19 infection is characterized by a “cytokine storm” leading to acute respiratory distress syndrome (ARDS) and

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respiratory failure.[2] An uncontrolled inflammatory response associated with marked pro-inflammatory cytokine release has been noted, resulting in decreased lymphocyte numbers and function, and granulocyte and monocyte abnormalities. The immune dysfunction induced by SARS- CoV-2 infection may result in secondary infections, septic shock, and multiple organ dysfunction.[3]

Vitamin D, a pluripotent steroid hormone, is essential for bone and mineral homeostasis.1,25-Dihydroxvitamin D_3 [$1,25(OH)_2D_3$] is the hormonally active form of this vitamin. Vitamin D status is reliably reflected by the serum $25(OH)D_3$ level. Apart from regulation of calcium metabolism, growth and proliferation, vitamin D has key roles in immune regulation, and the prevention of inflammation and autoimmunity. Various data showed that, apart from the modulation of innate immune cells, it also promotes immunological tolerance.[4] Calcitriol suppresses proliferation, differentiation of T helper (Th) cells and modulates their cytokine production. It also modulates proliferation and differentiation of B lymphocytes.[5] Moreover, vitamin D suppresses the production of the pro-inflammatory cytokines of the adaptive immune system (such as IL-1, IL-6, etc.), especially those involved in acute inflammatory responses like cytokine storm which is responsible for the mortality observed in COVID. Studies have shown that decreased levels were associated with the severity of respiratory infectious diseases such as bronchitis, pharyngo-tonsillitis, viral pneumonia.[6] Several epidemiological studies demonstrated that vitamin D deficiency is associated with increased severity of COVID-19 disease. In view of these facts, our study was done to find out the prevalence of vitamin D deficiency in COVID-19 patients and also to find out any relation of vitamin D deficiency with severity of COVID-19 disease. [7-12]

MATERIALS AND METHODS

This hospital-based cross sectional observational study was conducted at Medical College and Hospital, Kolkata during the time period from January 2021 to January 2022. 100 RTPCR confirmed COVID patients with moderate and severe disease based on clinical criteria were enrolled in this study after obtaining clearance from institutional ethical committee (IEC Clearance number: MC/KOL/IEC/NON-SPON/947/01/2021 dated 20/01/2021). Informed consent was taken from all subjects. We excluded patients of age below 12 years, pregnant women, subjects with chronic kidney disease and those who were already on vitamin D supplementation for any reason from our study population. Cases were classified as Mild, Moderate and Severe disease based on oxygen saturation (at rest) as per WB state government guideline. Patients with SpO2 $\geq$ 95% classified as Mild category; patients with SpO2 of 90-94% were Moderate category and <90% were severe disease category.

Blood samples were drawn from each subject within 24 hours of admission. Chemiluminescence immunoassay was used to determine the serum 25-hydroxyvitamin D [25(OH)D] concentration, which is the major circulating form of the vitamin, and the levels were categorized as normality ($\geq$30 ng/mL), insufficiency ($\geq$20- <30 ng/mL) and deficiency ($\leq$ 20 ng/mL). Complete hemogram including total leukocyte count, blood biochemistry including liver function test (SGOT, SGPT, serum albumin), renal function test, serum inflammatory markers like Erythrocyte Sedimentation Rate, C-reactive protein; D-Dimer, Lactate Dehydrogenase, ferritin were also assessed using standard laboratory methods. High Resolution Computed Tomography scan was performed in all subjects. CT severity score was calculated as per the extent of anatomic involvement in each of 5 lobes, as follows: no involvement- 0; < 5% - 1; 5–25%- 2; 26–50%- 3; 51–75%- 4; and > 75% involved- 5. Each individual lobar score was summed together to calculate the global CT score (0 to 25).[13] CT severity score of less than 7 was considered mild pulmonary involvement, score between 7 and 18 was moderate involvement and score of more than 18 was severe pulmonary involvement.

A structured proforma was used to collect data from the relevant subjects of the study. Data was entered into and analysis done using Statistical Packages for Social Sciences (SPSS) version 28.0. The categorical data were expressed as percentages and absolute numbers. The continuous numerical data were expressed in mean +/- SD. Chi squared test was used to test for significant difference of proportions (categorical data). Independent t-test and analysis of variance (ANOVA) were used to test for significant difference of means (continuous data). Also, Receiver Operating Characteristic (ROC) curves for statistically significant parameters were obtained to predict the severity of COVID-19 disease. All tests were analysed with 95% confidence interval with a P value of <0.05 considered significant.

Approval of Institutional Ethical Committee: Approved by the Institutional Ethics Committee, Medical College, Kolkata on January 27, 2021, IEC Clearance number: MC/KOL/IEC/NON-SPON/947/01/2021.

RESULTS

In the current study, we analysed 100 RTPCR or Rapid Antigen Test confirmed COVID-19 patients, out of which 53 and 47 subjects were male and female, respectively.

The mean age of the subjects in this study was 51.68 ± 13.68, range 22-81 years.

Among the 100 COVID-19 patients, 53 (53%) patients were suffering from moderate disease and 47 (47%) patients were suffering from severe disease.

In the current study, mean vitamin D level was 20.33 ± 8.939 ng/dl. Among 100 COVID-19 patients, 15 (15%) patients had normal vitamin D level. 18 (18%) and 67 (67%) patients were vitamin D insufficient and deficient, respectively.

Mean haemoglobin level was 11.81 ± 1.37 gm/dl. 11 subjects had haemoglobin level below 10 g/dl; 47 patients had levels between 10-12 g/dl and rest of patients had level

>12 g/dl. Mean value of serum albumin, ferritin and LDH was 3.319 ± 0.3366 g/dl, 430.61 ± 267.5 mcg/L and 694.52 ± 417.02 IU/L respectively.

In our study out of 100 patients, HRCT thorax severity score was <7 in 5% patients, whereas score of >18 was in 41% patients. 54% patients had CT severity score between 7-18. [Table no: 1]

Mean Vitamin D level in moderate disease category was noted to be 23.23 ± 8.74 ng/dl and in severe disease category was 17.17 ± 8.09 ng/dl. [Table no: 2]

In moderate disease severity group, 28 (52.83%) patients had Vitamin D deficiency, 14 (26.41%) subjects were Vitamin D insufficient and 11 (20.75%) subjects had normal Vitamin D level. In severe disease group, 39 (82.97%) subjects had Vitamin D deficiency, 4 (8.51%) subjects were Vitamin D insufficient and 4 (8.51%) subjects had normal Vitamin D level. Association of severity of disease and vitamin D deficiency was noted to be significant on statistical analysis. (p<0.05) [Table no:3]

Table 1: Distribution of HRCT Severity Score

HRCT Severity Score	Cases (n=100) (%)
<7	7 (7)
7-18	43 (43)
>18	50 (50)

Table 2: Vitamin D3 and Severity of Disease

Vitamin D3 deficiency Severity	Cases	Mean ± SD
Moderate	53	23.23 ± 8.74
Severe	47	17.13 ± 8.09

P value <0.001, significant (calculated using unpaired t test)

Table 3: Vitamin D deficiency and severity of disease

Severity of disease	Vitamin D deficiency status			Total
	Deficiency	Insufficiency	Normal	
Moderate	28 (52.83)	14 (26.41)	11 (20.75)	53
Severe	39 (82.97)	4 (8.51)	4 (8.51)	47
Total	67	18	15	100

Chi-Square value-10.305, P-value: 0.006**

Table 4: Vitamin D status and age and sex

Variable	Vitamin D status			P value (Chi square test)
	Deficiency (%)	Insufficiency (%)	Normal (%)	
Sex				
Male	38 (71.69)	9 (16.98)	6 (11.32)	0.480
Female	29 (61.70)	9 (19.14)	9 (19.14)	
Age (in years)				
<30	4 (66.67)	1 (16.67)	1 (16.67)	0.842
30-60	41 (66.13)	10 (16.13)	11 (17.74)	
>60	22 (68.75)	7 (21.88)	3 (9.38)	

P value <0.05 indicates statistical significance

Table 5: Biochemical, radiological parameters and vitamin D level

Parameters	Vitamin D3 status			P value (ANOVA)
	Normal (N=15)	Insufficiency(N=18)	Deficiency (N=67)	
Haemoglobin (Mean ± SD)	3.53 ± 3.02	5.28 ± 3.37	7.24 ± 5.95	0.034**
TLC (Mean ± SD)	7940.00 ± 2238.53	9771.06 ±2216.89	11642.99 ± 12882.02	0.441
NLR (Mean ± SD)	3.53 ± 3.02	5.28 ± 3.37	7.24 ± 5.95	0.034**
LDH (Mean ± SD)	536.47 ± 196.04	767.28 ± 733.58	710.36 ± 326.58	0.249
Ferritin (Mean ± SD)	286.13 ± 171.99	352.67 ± 252.46	483.90 ± 274.62	0.012**
CT Severity Score (Mean ± SD)	13.73 ± 5.54	14.44 ± 4.49	16.79 ± 4.95	0.042**

P value <0.05 indicates statistical significance

Table 6: Biochemical parameters and disease severity

Parameters	Disease Severity		P value
	Moderate (N=53)	Severe (N=47)	
N/L Ratio (Mean ± SD)	4.23 ± 2.76	8.70 ± 6.52	**<0.001***
ESR (mm/Hr) (Mean ± SD)	27.21 ± 10.86	39.47 ± 11.90	**<0.001***
CRP (mg/L) (Mean ± SD)	36.50 ± 31.42	73.22 ± 24.79	**<0.001***
SGOT (U/ml) (Mean ± SD)	28.43 ± 16.86	54.30 ± 30.48	**<0.001***
SGPT (U/ml) (Mean ± SD)	27.68 ± 16.83	46.02 ± 35.16	**0.001***
Albumin (gm/dl) (Mean ± SD)	3.49 ± 0.54	3.15 ± 0.42	**0.001***
D-Dimer (mcg/ml) (Mean ± SD)	0.91 ± 0.91	1.90 ± 0.68	**<0.001***
Ferritin (mcg/L) (Mean ± SD)	315.06 ± 212.96	560.91 ± 264.73	**<0.001***

*Statistically Significant, calculated using unpaired t test.

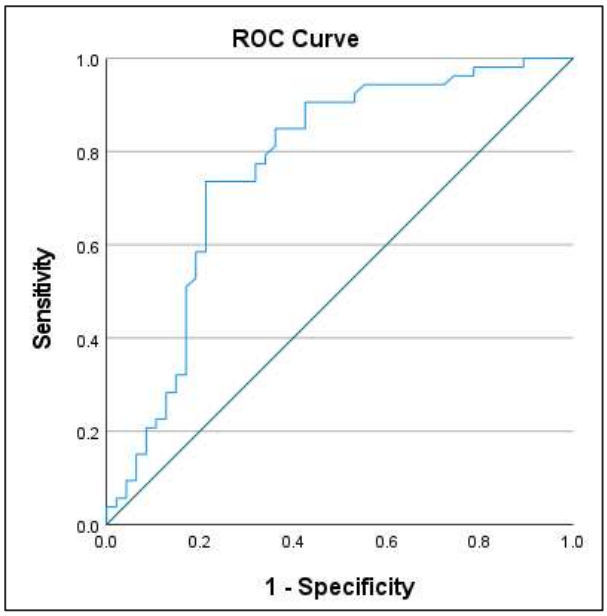

Figure 1: ROC curve showing sensitivity and specificity of vitamin D in predicting severity of disease

Table 7: Serum LDH and disease severity

Severity of Disease	LDH		Total
	Normal	Elevated	
Moderate	26 (49.05%)	27 (50.94%)	53 (100%)
Severe	4 (8.51%)	43 (91.48%)	47 (100%)
Total	30	70	100

Chi-Square value: 19.501, P-value: <0.001

Table 8: CT severity score and disease severity

Severity of disease	CT severity score			Total
	<7	7-18	>18	
Moderate	7 (100 %)	43 (100 %)	3 (6 %)	53
Severe	0	0	47 (94 %)	47
Total	7	43	50	100

Chi-Square value: 88.679, P-value: <0.001

In the current study, 62 (62%) subjects were in 30-60 years age group, 32 (32%) were of ages above 60 years old, and 6 (6%) were below 30 years old. Vitamin D deficiency was maximally prevalent (41% of total patients and 61.19% of all Vitamin D deficit patients) in age group between 30 to 60 years; followed by in age >60 years (22% of total patients and 32.88% of all Vitamin D deficit patients). Association between age and Vitamin D Deficiency status was not noted to be significant. (p-value: 0.842) [Table no: 4]

In our study, 29 (61.70%) female patients had Vitamin D deficiency and 9 (19.14%) female patients had Vitamin D insufficiency. Among male patients, 38 (71.69%) were deficient and 9 (16.98%) were Vitamin D insufficient. No statistical significance was found between gender and Vitamin D group. (p-value: 0.483) [Table no: 4]

The difference of TLC, serum LDH value in vitamin D groups was not statistically significant. But, haemoglobin level, N-L ration, serum ferritin value and CT severity score was statistically significantly different among vitamin D groups. [Table no: 5]

Receiver operator curve (ROC) analysis showed that vitamin D level would be predictive for severity of disease. The cut off value of vitamin D for predicting severe disease was found to be 18.57 ng/ml. (sensitivity of 73.6%; specificity of 78.7%; AUC: 0.768; 95%CI: 0.670-0.866; p-value<0.001) [Fig no: 1]

We also analyzed relationship between various biochemical parameters and severity of COVID-19 disease. N/L ration, ESR, CRP, liver enzymes, serum albumin, D-dimer and ferritin was statistically significantly associated with disease severity. [Table no:6]

In our study, 27 (50.94%) patients of moderate disease category had elevated serum LDH value; whereas in severe disease category, 43 (91.48%) patients had elevated LDH level. A Chi-square test was conducted between group of serum LDH and Disease Severity, and a statistically significant association was found. (p-value <0.001) [Table no: 7]

The mean CT Severity score in severe disease group was 20.34± 1.77 and in moderate disease group was 11.98± 3.57. We had found that CT severity group was statistically significantly associated with disease severity. (p-value<0.001) [Table no: 8]

DISCUSSION

In the current study, we analysed 100 RTPCR or Rapid Antigen Test confirmed COVID-19 patients. Among the 100 subjects, 53 (53%) were suffering from moderate disease and 47 (47%) were suffering from severe disease.

The prevalence of severe disease was maximum in patients with age between 30 to 60 years (59.57% of all severe patients). Disease severity was slightly more prevalent in female (48.93%) than male patients (45.28%). We could not find any association between age, gender and disease severity. (p-value>0.05)

In our study, among 100 COVID-19 patients, 15% of patients had normal vitamin D level. Vitamin D insufficiency was noted in 18% of patients, 67% had vitamin D deficiency. We found that, mean vitamin D level in moderate COVID-19 disease patients was 23.23±8.74 ng/ml and in severe disease patients, it was 17.17±8.09 ng/ml. So, in this study, the prevalence of vitamin D Deficiency was 67%. The prevalence of vitamin D deficiency in severe disease group was 82.97% and in moderate disease group it was 52.83%. Association between the severity of disease and vitamin D deficiency status was observed to be significant. (p<0.001)

Dieter De Smet et al conducted a study in March 2020 on the association between serum 25(OH)D levels at hospital admission and COVID-19 stage and mortality; they concluded that low 25(OH)D levels on admission are associated with COVID-19 disease severity and 59% were vitamin D deficient on admission and the result was in

concordance to our study.[12] Emanuele Cereda et al did a study in September 2020 including 129 patients (54.3% males, mean age 73.6 ± 13.9 years) where they found that 13.2%, 22.5% and 54.3% of patients were 25(OH) vitamin D insufficient, deficient and severely deficient, respectively.[14] A similar kind of result as that of our study, was found in a case-control study conducted by K Ye et al. In that study vitamin D deficiency was the greatest in severe/critical cases (80%), in comparison to mild cases (36%), with a statistically significant association between vitamin D deficiency and severe/critical disease ($p < 0.05$).[11]

The receiver operator curve (ROC) analysis showed that vitamin D level would be predictive for severity of disease. Cut off vitamin D level to predict severity of disease was found to be 18.57 ng/ml (sensitivity of 73.6%, specificity of 78.7%; AUC 0.768; 95%CI: 0.670-0.866; p-value<0.001). Teama MA et al. found that a Vitamin D value less than18 ng/ml could predict a poor prognosis with a sensitivity and specificity of 60.6% and 75.9%, respectively. The positive and negative predictive values were 74.1% and 62.9%, efficiency 67.7%, area under curve (AUC) of 0.783, with a p-value <0.001. [15]

We did not find any significant difference between age, gender and vitamin D deficiency. A statistically significant difference was found between N/L Ratio and vitamin D deficiency group (p-0.034). A significant association was found between Neutrophil-Lymphocyte Ratio and severity of COVID-19. (p-value <0.001) Chan AS et al. have found a similar result in their study. [16]

In current study, the mean level of ESR was 32 mm/hr. CRP was elevated in 88% of patients. 21% of patients had normal D-Dimer level and 79% of patients had elevated D-dimer. 46 (97.87%) Patients in the severe disease category had elevated D-dimer level and 33 (62.26%) patients with moderate disease severity had elevated D-Dimer levels. 28% Of patients had elevated levels of SGOT and SGPT levels was elevated in 33% of patients. Out of 100 patients, 28% had elevated serum urea levels and 6% had raised serum creatinine levels. Study of Cheng Y et al. showed similar renal impairment in COVID. [17]

We observed the severity of COVID-19 to be statistically significantly associated with ESR, CRP, D-Dimer, serum SGOT, SGPT level (p-value<0.001). The mean serum albumin level of patients (mean ± SD) was 3.319 ±0.3366 g/dl. Serum albumin level and disease severity were found to be associated in a statistically significant manner. (p-value<0.001)

Y Wang et al. demonstrated that albumin values in COVID-19 patients varied significantly with varying degrees of disease severity. Also, studies have suggested that patients with COVID-19 may have liver damage as indicated by the presence of elevated alanine aminotransferase and aspartate aminotransferase values. [18]

The study by Y Yao et al. concluded that D-dimer levels correlated with the severity of COVID-19 disease and served as a reliable prognostic marker for in-hospital mortality in hospitalised patients. [15,19]

The mean value of serum ferritin between vitamin D deficiency groups were found to be statistically significant. We also found significant association of COVID severity with serum ferritin level (p-value<0.001). Lin Z et al. concluded in their study that the on-admission serum ferritin level serves as an independent risk factor for disease severity in COVID-19 patients. [20] Anshul Jain et al. performed a study in the year 2020 to analyse the vitamin D level in COVID-19 patients and its effect on disease severity and found that the serum level of ferritin was higher in patients with vitamin D deficiency. [8]

We observed that 50.94% of patients in the moderate disease category had an elevated serum LDH value; whereas in the severe disease category 91.48% of patients had an elevated LDH level and the association was statistically significant (p-value <0.001). Chang Li et al. noted that the on-admission serum LDH was useful in evaluating the disease severity and in-hospital mortality among patients with COVID-19. [21]

We found that the CT severity group was statistically significantly associated with disease severity (p-value<0.001) and a negative correlation was observed between vitamin D level & CT severity score (p-value<0.001). Teama MA et al. concluded in their study that lower vitamin D levels were significantly associated with increased disease severity (p-value < 0.001), greater duration of disease, higher serum inflammatory markers (including D-dimer, CRP, and ferritin), and a higher CT Severity Score. [15]

Limitations: Our study was conducted on a small population of patients in a short time period; long term multicentric studies are warranted are necessary in this field. Mild cases were excluded from the study and most of the cases were collected during the first wave of COVID 19, which may have caused clustering of cases.

CONCLUSION

From this study, we concluded that vitamin D deficiency is strongly associated with the severity of COVID-19 disease. Low serum vitamin D levels can be predictive of severe COVID-19 disease. Severity of COVID-19 disease is strongly associated with elevated inflammatory markers (ESR, CRP, serum ferritin), high neutrophil-lymphocyte ration, low serum albumin, elevated LDH level and greater CT severity score.

Authors' Contributions: **EH**- Study conception, design, data collection, analysis, manuscript preparation; **SKM**- Study conception, design, analysis, manuscript preparation and revision; **SS**- Data collection, analysis and interpretation, manuscript preparation; **AJ**- Data analysis, manuscript revision and editing; **RM**- Manuscript revision

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