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Research Article

Vitamin D Levels and its Association with Inflammatory Markers, Severity and Outcome in Hospitalised COVID-19 Patients - An Indian Perspective

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I N F O

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A B S T R A C T

Background: The recent COVID-19 pandemic has taken over the world in enormous proportions like none other in recent times. Vitamin D plays an essential role in the immune system and has proven to have importance in the context of many respiratory infections. This study aims to unveil the possible correlation between vitamin D levels and disease outcome and affirm the role of hypovitaminosis D as an independent risk factor for severity in COVID 19.

Methods: We studied 209 RTPCR confirmed COVID 19 positive cases. Serum 25(OH) D levels were done at the time of admission. HRCT chest and inflammatory markers (serum ferritin, D dimer, IL6 and CRP) were estimated on admission and repeated on case-to-case basis.

Results: Out of 209 patients studied, 44.49% had vitamin D deficiency, 24.40% had Vitamin D insufficiency, and 31.11% had normal Vitamin D levels. Vitamin D deficiency significantly increased with advancing age. The mean vitamin D level in our study is 27.47 ± 21.86. 27.8% had mild COVID-19 disease as evidenced by HRCT imaging, 21.1% showed moderate disease, and 51.2% showed severe COVID-19. The mean values of inflammatory markers in Vitamin D deficient patients were as follows: D dimer (3298.88 ± 2230.14), Serum Ferritin (530.88 ± 497.14) CRP (175.34 ± 87.27) IL6 (125.39 ± 275.72). Significant correlation of Vitamin D with C reactive protein ($r = -0.18$, $p \leq 0.05$), D-Dimer ($r = -0.42$, $p < 0.0001$), and CT severity score ($r = -0.24$, $p \leq 0.0001$) was noted.

Conclusion: In our study, Vitamin D inversely proportional levels were inversely proportional to CRP, D-Dimer, and CT severity.

Keywords: Vitamin D, COVID19, Inflammatory Markers

Introduction

The recent COVID-19 pandemic has taken over the world in enormous proportions like none other in recent times. Known for its remarkable variance in clinical severity, COVID-19 is characterised by the presence of pneumonia, acute respiratory distress syndrome,thrombotic events, myocarditis, and cytokine storm for which the underlying pathology is inflammation.[1]

SARS-CoV-2 is prone to evolve genetically due to its ability to adapt to its human host. Hence, overtime it can mutate and have characteristics that differ from its parent virus. Till now four mutations of SARS-CoV-2 are known which are Alpha, Beta, Gamma and Delta.SARS-CoV-2 is a Novel Beta coronavirus having a round or an elliptical shape.[1]

It is always debated that host constituents can impact consequences. With co-morbidities like diabetes mellitus, coronary artery disease, obesity, and smoking being linked to clinical outcomes, insufficient levels of various micronutrients can also pose an independent risk factor.[2]

Known to have a key role in calcium and phosphorous metabolism, vitamin D has recently been postulated to play a central role in the modulation of the immune-related responses in both infectious and autoimmune diseases.[2] Vitamin D has a prime role in the expression of angiotensin-converting enzyme 2, which is an important mediator in the pathogenesis of COVID19 infection. In the past, with reference to MERS and SARS COV-1 which has caused illnesses affecting the Lungs and Airways, Calcitriol is known to control the immune mechanisms, pathogenesis, inflammation and modulatory reactions.[3]

In ARDS caused by SARS-CoV-2, its phases comprising viremia initially and increased inflammation later can be targeted by products of metabolism of calcitriol in the host. Reactions by Calcitriol included induction of proteins against the microbe and programmed cell death.[3]

Hence, this study aimed at measurement of Vitamin D levels and its correlation with clinical severity, CT severity score and inflammatory markers in COVID-19. It also aimed at observing the distribution of baseline characteristics and comorbidities amongst the study population. We wanted to further assess if low calcitriollevels cause severe COVID-19 with a catastrophic coursethatneeded further investigation.

Materials & Method

This is a cross-sectional, single centre study carried out in semi-urban south Indian population from October 2020 to October 2021 at Chettinad Hospital and Research Institute, Kelambakkam. The study included 209 patients.

Patients not willing to give the informed consent were excluded from the study.

The sampling technique employed was simple random sampling.

Sample Size Calculation

n = $(Z1\text{-alpha}/2)^2$ $(P\{1\text{-}P\}/D^2)$, where Z 1-alpha = Z 0.05, means1.96, 95% Confidence interval (CI), P= 15%(0.15), and D = 5% (0.05). Therefore, n = 1.96^2 x $(0.15(1\text{-}0.1)/0.05)^2$= 196 and taking 10% nonresponse rate the sample size was decided to be 209 patients.

Inclusion Criteria

All subjects fulfilling the WHO case definition of COVID-19[4] and COVID-19 cases detected by RT-PCR investigation were included in the study after giving informed consent.

Indian Council of Medical Research recommended case definitions[5] were incorporated for defining the clinical severity of COVID-19:

Mild illness: Fever with or without upper respiratory tract symptomatology barring breathing difficulty/decreased oxygen saturation.

Moderate illness: Individuals having either features: tachypnoea (RR>24/min), breathing difficulty or SpO_2between 90-93 percent without any oxygen support.

Severe disease:Patients having anyone of the following features, eitherrespiratory rate >30/min, breathlessness or SpO_2< 90% on room air.

Serum 25 (OH)D estimation levels were performed on admission along with basic investigations including complete haemogram, blood biochemistry, inflammatory markers like D Dimer, CRP, IL6, and serum ferritin were done on admission and repeated as required on a case-to-case basis. The highest levels of inflammatory markers were included in the study for correlation with serum 25(OH) levels.

Vitamin D levels were estimated using chemiluminescent immunoassay (CLIA) method. On basis of multiple guidelines, Vitamin D levels were classified as follows:[6]

- Vitamin D deficiency: Serum (OH) D levels < 20ng/mL
- Vitamin D insufficiency: Serum (OH) D levels between 20-30 ng/mL
- Normal vitamin D levels: Serum (OH) D levels more than 30ng/mL

Phillips brilliance 128 slice CT machine was used for high resolution computed tomography chest imaging done with the patient in supine posture and breath held when they presented with symptoms initially. HRCT was repeated if required on a case-to-case basis.CT severity score was calculated and was classified as follows.[7]

- Mild disease: Score of 7 or less
- Moderate disease: Score of 8-17
- Severe disease: Score of more than 18 (up to 25)

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Ethical Issues/ Clearances

Institutional ethics committee clearance was obtained before the start of the study. Informed consent was obtained from all participants included in the study.

Statistical Analysis

Data wereentered using Microsoft Excel 2013. Qualitative variables given in percentage and frequency and quantitative variables given in mean with standard deviation were analysed by Windows SPSS software version 26.The distribution of continuous variables was normal. For nonparametric statistics, Windows SPSS software version 26 was utilised for statistical analyses. Pearson correlation coefficient was used to find the correlation between quantitative variables. Bar diagrams and pie charts were used to represent the data. Less than 0.05 p value was taken with statistical significance.

Results

A cross-sectional study was carried out among 209 hospitalised COVID-19 patients in our institution. The mean age in years of the study participants was 56.41±16.08 years. The study was conducted on 158 males (75.6%) and 51 females (24.4%). The mean duration of hospital stay in days was 9.70± 6.15 for patients with vitamin D deficiency. Comorbidities noted are mentioned in Table 1. The most common comorbidity noted amongst the study participants was type 2 diabetes. Presenting symptoms of the study population included, 75.1% had fever, 48.3% had cough, 48.3% had breathlessness,33.5% had myalgia, 17.2% had common cold,10% had loose stools, 9.1% had headache 5.3% had loss of taste and 1.4% had anosmia. In our study, 43.7% received remdesivir, 75.6% received LMWH and 56.4% were administered dexamethasone where as 19.6% were on prednisolone.Vitamin D deficiency significantly increased in frequency with advancing age (p=0.006).In this current study, a significant correlation was observed between vitamin D deficiency and total protein (p=0.0001) and globulin (p ≤ 0.0001). The baseline characteristics of the patients are shown in Table 1.

Table 1.Baseline Characteristics

	Frequency	Percentage
<50 yrs	61	29.2
50-60 yrs	49	23.4
>60 yrs	99	47.4
Females	51	24.4
Males	158	75.6
Diabetes	100	47.8
Hypertension	68	32.5
CKD	11	5.3
CVA	5	2.4
CLD	2	1
CAD	24	11.5
BA-COPD	9	4.3
OLD PTB	1	0.5
Hypothyroid	14	6.7

Distribution based on vitamin D levels in the present study shows 44.49% had vitamin D deficiency, 24.40% had vitamin Din sufficiency, 31.11% had normal vitamin D levels. The mean vitamin D level in the current study was 27.47±21.86ng/mL.

Table 2.Vitamin D Levels

Levels	Severity	Frequency	Percentage
1-20	Deficiency	93	44.49
20-30	Insufficiency	51	24.40
More than 30	Normal level	65	31.11

In our study, illness of high severity was present in 91.3% of 25(OH) D deficient individuals whereas illness of mild severity was present in 0.03% of 25(OH) deficient individuals. Hence, vitamin D levels significantly correlated with clinically severe disease with a p value of equal to 0.01.

The vitamin D levels and clinical severity is shown in Table 3.

Table 3.Vitamin D Levels and its Correlation with Clinical Severity

		Vitamin D Levels		
Clinical Severity		Less than 20 (mg/mL)	20-30 (mg/mL)	More than 30 (mg/mL)
	Mild disease	2	10	63
	Moderate disease	6	35	1
	Severe disease	85	6	1

Table 4.Vitamin D and its correlation with inflammatory markers

Vitamin D levels	Mean ± SD	P value
D-Dimer and Vitamin D		
<20	3298.88 ±2230.14	F value = 27.44 P ≤ 0.0001*
20- 30	1549.82 ±1771.55	
>30	1139.45 ±1465.26	
*Significant co-relation at p value ≤ 0.05		

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IL6 and Vitamin D		
<20	125.39 ± 275.72	F value = 0.64 P= 0.52
20-30	135.20 ± 302.82	
>30	185.25 ± 352.41	

Serum Ferritin and Vitamin D		
<20	530.88 ± 497.14	F value = 0.47 P= 0.62
20-30	466.67 ± 381.20	
>30	550.65 ± 477.17	

CRP and Vitamin D		
<20	175.34 ± 87.27	F value = 10.68 P ≤ 0.001*
20-30	105.73 ± 75.66	
>30	131.78 ± 64.56	
*Significant co-relation at p value ≤ 0.05		

In our study, 85 individuals with severe COVID-19 disease had Vitamin D levels of less than 20.

The correlation of inflammatory markers and Vitamin D levels are shown in Table 4.

The mean values of the inflammatory markers of COVID-19 and its correlation with vitamin D levels are shown in Table 3. The current study showed a significant correlation of vitamin D with C reactive protein (r=-0.18, p ≤ 0.05*) and D-Dimer (r=-0.42, p<0.0001*) (*Significant co-relation at p value ≤ 0.05)

The correlation between vitamin D levels and CT severity score has been shown in Table 5.

Table 5.CT Severity Score and its Correlation with Vitamin D

CT Severity	Frequency	Percentage
Mild (CT Severity Score 0-7)	58	27.8
Moderate (CT severity score 8-18)	44	21.1
Severity (CT severity score 18-25)	107	51.2

Vitamin D levels	CT Severity (Mean ± SD)	P value
<20	18.74 ± 7.38	F value = 4.59 P= 0.01*
20-0	15.11 ± 8.11	
>30	14.06 ± 7.22	
*Significant co-relation at p value ≤ 0.05		

In our study, 27.8% had mild COVID19 disease as evidenced by HRCT imaging, 21.1% showed moderate disease and 51.2% showed severe COVID-19. The current study showed a significant correlation of vitamin D with CT severity score (r=-0.24, p ≤ 0.0001*). (*Significant co-relation at p value ≤ 0.05)

Table 6.Pearson Correlation Test between Vitamin D Levels and Clinical Severity

Cross - Tabulation					
		Vitamin D			Total
		<20	20-30	>30	
Clinical Severity	Mild disease	2	10	63	75
	Moderate disease	6	35	1	42
	Severe disease	85	6	1	92
Total		93	51	65	209

Pearson Correlation

Pearson Correlation			
		Clinical Severity	Vitamin D
Clinical Severity	Pearson Correlation	1	-0.893
	Sig. (2-tailed)		0.000
	N	209	209
** Correlation is significant at the 0.01 level (2-tailed).			

Hence, the study showed a significant correlation between low Vitamin D levels and severe COVID-19 disease.

The mortality in our study was 47.8%as depicted in Table 7.

Table 7.Mortality

	Frequency	Percentage
Recovered	109	52.2
Succumbed	100	47.8

Discussion

Vitamin D interferes with the functioning of immune system and the cells mediating it like macrophages, T lymphocytes, dendritic cells, monocytes and macrophages. IL-1β and TNFα are the key activators for the secretion of the IL-6.[16]Vitamin D3 has been recommended in COVID-19 patients to decrease the severity and also for prevention of infection.[17]In the present study, the mean age of the patients was 56.4±16.08 (years) and there was a significant association between vitamin D deficiency and advancing age. This was in consonance with the observations made by CarpaganoGE et al.[8] and RadujkovicA et al.[9]

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It was observed that a higher proportion of patients (70.3%) were found to be deficient in Vitamin D. 15% of the patient had vitamin D levels <10 (ng/dl) and 30.1% had Vitamin D <20 (ng/dl) The median Vitamin D level was 22.43± 21.8 (ng/dl).

Demir M et al.[11] in their study reported that vitamin D levels were below 30 ng/mL in 94.27% of 227 COVID-19-positive patients while 93.07% of 260 non-COVID-19 patients had vitamin D levels below 30 ng/ml. A similar observation was done in the study done by RadujkovicA et al.,[8] D'avalioA et al.[11] Research considering cross-section of population of COVID-19 patients of Indian origin has told 58.97% deficient calcitrioland 89.1% insufficient calcitriol.[13]

A recent meta-analysis by Munshi R et al.[12] concluded that the levels of Vitamin D can be used as a prognostic indicator to assess the severity of COVID-19, but it was always quite debatable to decide the cut off values of vitamin D levels.

In the present study, it was observed that severity of the disease is more in cases with vitamin D deficiency wherein 40.86% of the patients with vitamin D deficiency have succumbed to the disease. Nimavat N et al.[13] reported in their study that calcitriol levels were directly proportional to severe illness. Calcitriol was deficient in 37.1% with severe illness, 10.1% with moderate illness and 20% with mild illness. In our study, vitamin D levels were significantly associated with CRP levels (r= -0.18, p less than 0.05). They also correlated with severity score by CT (r=-0.24, p less than 0.0001). They were also correlated with D-dimer levels (r=-0.42, p value less than 0.0001).

Amongst COVID-19 critical cases, an increased percentage of patients had severely deficient calcitriol levels as compared to COVID-19 cases of mild severity in a study given by Ye Ket al.[14]Vitamin D deficiency can be correlated to overall nutritional deficiency as evidenced by a concomitant decrease in the total protein levels also. This observation made was similar to the study done by Carpaganoet GE etal.[8]

In consonance with Ricci A et al.[18] and other individual studies as well as meta-analyses, the mean CT severity of patients having vitamin D deficiency was18.74 ± 7.38, and patients with vitamin D insufficiency was15.11 ± 8.11, indicating a higher CT involvement in patients with vitamin D deficiency.

In the current study, vitamin D was observed to be inversely proportional to D Dimer($p \leq 0.001$) and CRP($p \leq 0.001$). Vitamin D deficient patients showed mean D Dimer levels (3298.88 ± 2230.14). Mean C- reactive protein levels of 175.34 ± 87.27was observed in patients with vitamin D deficiency, similar toresults published by Sunnetcioglu A et al.[19] The mean levels of serum Ferritin (p-0.54) and IL-6 (p-0.30) were not observed to be in proportional to the severity of vitamin D deficiency. Though a significant association has been observed with both clinical and radiological severity, no association has been found in relation to mortality due to COVID-19.

Limitations

The study has a certain limitation of being a single centre study. To elicit associations with all the observations, much more elaborate and larger studies are to be conducted further. The results of the study cannot be generalized to the entire population.

Conclusion

In our study, low vitamin D levels were inversely proportional to CRP, D-Dimer and CT severity.Vitamin D deficiencycan thus be correlated with the clinical and radiological severity of COVID-19 and can be considered as an independent risk factor.

Further studiesare required to assess whether low vitamin D levels and comorbidities in COVID-19 disease determine its severity.

Conflict of Interest: None

Sources of Funding: None

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