Original Article

Comorbidities in COVID-19 Patients : Are these associated with Vitamin D Deficiency and SARS-CoV-2 Infection Grade ?

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Abstract

Background : Vitamin D, known for its immune benefits, is vital for bolstering defences. Evidence links COVID-19 comorbidities to low Vitamin D. Our goal is to assess COVID-19 patients, examining demographics, lab data, and post-infection comorbidities, focusing on vitamin D deficiency impact.

Materials and Methods : This was a cross-sectional study. Estimation of serum 25(OH)D was done in conjunction with other blood tests, including D-dimer and Complete Blood Count. All COVID-19 positive patients were checked for other health issues and medical emergencies. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) Version 23 for Windows. The demographic variables, COVID-19 severity, Vitamin D level, and comorbidity were calculated in numbers and percentages. The ANOVA test was used to find significant differences in Vitamin D, D Dimer to COVID severity.

Results : Fifty patients who were clinically diagnosed with positive COVID-19 by RT-PCR were included in this study. 74% (n=37) of patients were Vitamin D deficient. Eight per cent of patients (n=4) were diagnosed with insufficient Vitamin D levels, and 18% of patients had adequate Vitamin D levels. It was noted that after acquiring SARS-CoV-2 infection, 62% (n=31) were Diabetic, 36% (n=18) were Obese and 42% (n=24) patients were suffering from Hypertension. Other medical conditions, such as NS (20%), TH (6%), TB (4%), CKD (2%), and COPD (2%) were observed.

Discussion : Correlation was observed in the severity grade of COVID-19 infection and comorbidities. Moreover, the positive correlation between the laboratory and demographic markers was also observed.

Conclusion : SARS-CoV-2 infection had an impact on individuals' medical health. Health comorbidities were associated with the COVID-19 severity. Plus, our study demonstrated that lower Vitamin D levels also had a significant impact on demographic markers indicative of low Vitamin D levels that may be associated and responsible for infection severity and comorbidities.

Key words : Vitamin D, Comorbidities, COVID-19, Correlation of Infection Severity, Association with Vitamin D.

The COVID-19 pandemic has presented an unprecedented Global health challenge, prompting intensive research to unravel the multifaceted factors influencing disease severity and outcomes. Among these factors, the role of Vitamin D deficiency has emerged as a critical and intriguing avenue of investigation. Vitamin D, renowned for its immunomodulatory properties, is implicated in the intricate interaction between the immune system and various comorbidities that heighten the risk of severe COVID-19 manifestations[1].

Editor's Comment :

- The COVID-19 pandemic has exposed various vulnerabilities in human health, focusing on the influence of vitamin deficits on illness penalties.
- Vitamin D has garnered significant interest due to its crucial role in immune regulation and its potential impact on the severity of COVID-19.
- Addressing their deficiencies through dietary interventions, supplementation, and public health measures is essential for improving health outcomes during the pandemic and beyond.

Comorbidities such as Diabetes Mellitus (DM), Obesity, Nephrotic Syndrome (NS), Thyroid Disorders (TH), Tuberculosis (TB), Chronic Kidney Disease (CKD) and Chronic Obstructive Pulmonary Disease (COPD) have been identified as significant contributors to the vulnerability of individuals to severe COVID-19 complications. This paper reviews the

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existing literature to delineate the intricate connections between Vitamin D deficiency and these diverse comorbidities, shedding light on the potential mechanisms that underpin their collective impact on the course of COVID-19[2-5].

By exploring the nexus between Vitamin D, comorbidities, and COVID-19 infection grade, this research aims to provide a comprehensive understanding of the immunological dynamics at play, laying the groundwork for targeted interventions and therapeutic strategies to mitigate the severity of COVID-19 in individuals with underlying health conditions.

In the present study, we aim to understand how comorbidities post-COVID-19 infection and deficiency of Vitamin D are associated with infection grades. This study might open avenues for designing therapeutic strategies or lines of treatments for COVID-19 sufferers.

Materials and Methods

The present study adopted a cross-sectional design spanning a duration of six months, with approval obtained from the Institutional Ethical Committee. Informed consent was diligently obtained from all participating patients.

In this investigation, we assembled a cohort consisting of 880 individuals who tested positive for COVID-19 and presented with concomitant Vitamin D deficiency. From this cohort, a targeted subset of 50 participants was purposively selected for an in-depth methylation study, with a particular focus on exploring the interplay between comorbidities and disease severity in relation to Vitamin D deficiency which required good quality of extracted DNA to perfume the experiment. The selection criteria for this subgroup encompassed a comprehensive assessment of comorbid conditions and disease severity levels, ensuring a nuanced representation of the diverse clinical spectrum observed in COVID-19 cases with Vitamin D deficiency. The investigation methodology adhered to established ethical guidelines and the selection process was designed to provide a robust foundation for examining the intricate relationships between Vitamin D status, comorbidities, disease severity and epigenetic modifications. The systematic approach employed in participant identification and subsequent methylation analysis aimed to unravel potential associations and shed light on the underlying molecular mechanisms governing the intricate relationship between Vitamin D deficiency, comorbidities and COVID-19 severity. The statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences) Version 23 for Windows. Demographic variables, COVID-19 severity, Vitamin D levels, and comorbidities were computed in both absolute numbers and percentages. The ANOVA test was employed to identify significant differences in Vitamin D, D Dimer and their association with the severity of COVID-19.

The study exclusively focused on clinically diagnosed COVID-19-positive individuals confirmed through RT-PCR testing. Following the World Health Organisation (WHO) standards, patients were categorised based on the severity of symptoms, distinguishing between mild, moderate, and severe cases[6].

To ensure the specificity of the study, individuals with a history of severe illnesses such as Cancer, Respiratory disease, Gastrointestinal disease and Kidney disease were excluded. Pregnant women, patients on Vitamin D supplementation and those with missing data were also omitted from the analysis.

Sample collection and testing were conducted at the Biochemistry Centre Clinical Laboratory situated at BJ Government Medical College and Sassoon General Hospital in Pune. Plasma 25-hydroxyvitamin D (25(OH)D) levels were measured using ELISA (kit name). In addition to Vitamin D levels, various laboratory parameters, including D dimer, Complete Blood Count, Liver Function Tests and Kidney Function Tests, were observed.

According to established guidelines, individuals with serum 25(OH)D levels below 20 ng/ml were classified as vitamin D deficient. The data analysis was carried out using SPSS software version 23. To discern significant differences among the study groups, the Analysis of Variance (ANOVA) test was employed. This rigorous methodology ensures the reliability and validity of our findings, contributing valuable insights into the association between Vitamin D deficiency and the severity of COVID-19 symptoms.

Results

This study comprised 50 individuals diagnosed with COVID-19 through RT-PCR. The majority of participants were male, with a male-to-female sex ratio of 2.1:1. The average age was 46.78 years, with

a Standard Deviation of ±22.10. Additionally, three newborns aged 1-2 weeks were included in the study population. SARS-CoV-2 severity-wise classification revealed that 32% of the patients fell within the mild category, while 38% were classified as moderate to severe cases. The remaining 32% presented with severe illness. Vitamin D deficiency, defined as serum levels below 20 ng/ml, was prevalent in a substantial majority, accounting for 74% of the patients.

Notably, 60% of the patients were identified as Obese. Diabetes Mellitus emerged as the predominant comorbidity among the study population, with 62% (n=31). Moreover, Neurological disorder (n=10) in 20%, Cancer in 18% (n=9), TH in 6% (n=3), TB in 2% (n=4) were dominantly found. Additionally, CKD, COPD, SLE, CCF and HIV were observed in 2% of patients, respectively (Table 1).

The severity of COVID-19 was observed in individuals with Diabetes Mellitus and Hypertension; the association did not achieve statistical significance. However, a statistically significant difference in age concerning COVID-19 severity was noted (P<0.05), indicating age as a potentially influential factor in the progression of the disease (Table 2). Furthermore, a positive correlation between Vitamin D levels and BMI was identified, although this correlation did not reach statistical significance.

Contrastingly, no statistically significant differences were found in Vitamin D levels or D dimer concerning COVID-19 severity (P>0.05). However, the relationship between vitamin D and other parameters can be potentially explored with a large population size (Table 3). These findings shed light on the demographic and clinical characteristics of the COVID-19 patients under investigation, providing valuable insights into the prevalence of comorbidities and vitamin D deficiency within this cohort (Table 4).

Table 1 — *Comorbidities according to the Vitamin D levels : This table shows the association between the Vitamin D levels and comorbidities. Patients with deficiency with Vitamin D seem to have more in number after acquiring the COVID-19 infection were associated with high risk factor comorbidities*

Parameters	Vitamin D (ng/ml) (%)				P Value
	≥30 (n=9)	20-30 (n=4)	10-20 (n=26)	<10 (n=11)	
Sex (M/F)	6/3	1/3	18/8	9/2	
Cancer	2 (22.2)	1 (25)	3 (11.5)	3 (27.3)	0.66
Diabetes Mellitus	6 (66.7)	3 (75)	13 (50)	9 (81.8)	0.29
Hypertension	6 (66.7)	2 (50)	12 (46.2)	4 (36.4)	0.18
NS	1 (11.1)	0	6 (23.1)	3 (27.3)	0.58
Obesity	4 (44.4)	2 (50)	9 (34.6)	3 (27.3)	0.80
Thyroid	0	0	1 (3.8)	2 (18.2)	-
COPD	0	0	1 (3.8)	0	-
Tuberculosis	0	0	2 (7.7)	0	-

Table 2 — *Comparison of comorbidity according to COVID severity*

Comorbidity	COVID severity (%)			Chi-square*	P Value
	Grade I (n=19)	Grade II (n=15)	Grade III (n=16)		
DM	10 (52.63)	9 (60)	12 (75)	1.78	0.18
HTN	7 (36.84)	8 (53.33)	9 (56.25)	1.33	0.25
Obesity III	8 (53.33)	13	9 (56.25)	7.62	0.11
	2	0	2		

*Linear by linear

Table 3(A) — *Correlations of Vitamin D levels with Laboratory markers, Age and BMI*

Correlation between Vitamin D and	r_s Value*	P Value
D Dimer	-0.08	0.57
WBC	0.306	0.031
Lymphocyte	-0.22	0.13
Age	-0.07	0.64
BMI	0.262	0.066

*Spearman rank correlation

Table 3(B) — *Correlations of COVID-19 severity grade with Laboratory and Demographic parameters*

Parameters	Grade I *versus* Grade II	Grade I *versus* Grade III	Grade II *versus* Grade III
VIT D (ng/mL)	0.60	1	1
D Dimer (mg/L)	1	1	1
WBC	1	1	1
Lymphocyte (%)	1	1	1
Age (Years)	0.012	0.14	1
BMI	0.24	1	0.89

By post hoc Bonferroni test: There is no significant difference of laboratory markers, age, BMI in Group I *versus* Group III, Group II *versus* Group III and Group I *versus* Group II except age as P>0.05. Group II had significantly more age than Group I as P<0.05.

Discussion

The observed increased trend in COVID-19 severity among individuals with Diabetes Mellitus[7,8] and Hypertension[9,10] while not achieving statistical significance, implies a potential association that warrants further investigation. These comorbidities have been previously identified as risk factors for severe outcomes in COVID-19, aligning with broader epidemiological patterns. The lack of statistical significance in this study may be attributed to the relatively small sample size, emphasising the need for larger cohorts to elucidate these relationships more definitively.

The significant difference in age concerning COVID-

Table 4 — *Comparison of Laboratory markers, Age and BMI with COVID severity*

Parameter	COVID severity						F Value	P Value
	Grade I (n=19)		Grade II (n=15)		Grade III (n=16)			
	Mean	SD	Mean	SD	Mean	SD		
VIT D (ng/mL)	17.57	10.089	13.30	8.776	15.02	9.418	0.87	0.42
D Dimer (mg/L)	1480.6	2149.6	2259.8	2769.7	1937.4	2658.7	0.41	0.66
WBC	9.55	6.113	11.08	7.545	10.09	6.218	0.23	0.80
Lymphocyte (%)	16.68	12.625	18.45	13.075	20.04	14.252	0.28	0.76
Age (Years)	35.76	18.708	57.22	21.527	50.06	21.681	4.87	0.012
BMI	29.99	3.972	32.10	2.445	30.81	3.438	1.61	0.21

19 severity highlights the pivotal role of age as a determinant of disease progression. Advanced age has consistently been acknowledged as a critical factor influencing the severity of respiratory infections, including COVID-19[11,12]. This finding aligns with existing literature and underscores the importance of age-stratified analyses in understanding disease dynamics.

Contrary to expectations, no statistically significant differences were found in Vitamin D levels and D dimer with respect to COVID-19 severity. While Vitamin D deficiency has been implicated in immune modulation, the absence of a significant association in this study suggests that the role of Vitamin D in COVID-19 severity may be more complex and multifactorial, warranting further exploration.

The positive correlation between Vitamin D levels and BMI, although not statistically significant, introduces an intriguing avenue for exploration. The relationship between Obesity, Vitamin D and COVID-19 outcomes is complex, with potential implications for immune function. The lack of statistical significance may be attributed to the need for more refined methodologies or larger sample sizes. The present literature has evidence for an association between Vitamin D, as a potent immunomodulator and SARS-CoV-2 infection severity, which underscores the potential role of Vitamin D in influencing immune responses. This connection provides a basis for exploring targeted interventions to optimise Vitamin D levels, potentially impacting the course of COVID-19. However, the association between Vitamin D and COVID-19 severity is complex and requires investigation at the genetic and molecular levels[13-16]. Our study also finds Cancer cases post-COVID-19 infection in Vitamin D-deficient patients. The detection of Vitamin D deficiency in Cancer cases raises intriguing questions about its potential role in cancer development or progression. Further research is warranted to elucidate the complex relationship between Vitamin D status and Cancer, offering insights that may inform preventive strategies and complementary therapeutic approaches[17,18].

The relationship between Vitamin D deficiency and COVID-19 is complex and multifactorial. Several factors contribute to Vitamin D deficiency in COVID-19 patients, such as limited sun exposure, underlying health conditions, an inflammatory response, reduced outdoor activities and malabsorption. Understanding these interconnections is crucial for developing strategies to address Vitamin D deficiency in COVID-19 patients. Supplementation, dietary adjustments, and recommendations for safe sun exposure are avenues that may be explored to optimise Vitamin D levels and potentially mitigate the severity of COVID-19 outcomes. Biesalski, *et al* in 2020, concluded that Vitamin D levels are associated with Vitamin D levels, which was matched with our results presented in subsequent tabular forms. Therefore, it's important to note that all comorbidities from the past COVID-19 infection might have a relationship with depleted levels of Vitamin D[19].

In conclusion, Vitamin D levels are associated with comorbidities and COVID-19 infection severity. Our study results offer valuable insights into the interaction of comorbidities, age and biomarkers in the context of COVID-19 severity. The first limitation of the study was relatively small sample size due to expertise performing the actual experimentation and higher cost of methylation study. Second, Vitamin D may be more important in combination with other nutrients also known to interact with the epigenome. Moreover, present manuscript only investigates the relation between COVID-19 infection severity with Vitamin D levels and comorbidities. Percent methylation, epigenetic modification or gene expression will be the future aspects of the study and are under examination. Finally, this was a cross sectional study; thus, no causal inference can be established. Future longitudinal studies including placebo-controlled

supplementation studies are needed to establish causality.

Conclusion

The SARS-CoV-2 infection had an impact on individuals' medical health. Health comorbidities were associated with the severity of COVID-19. Moreover, our study demonstrated that lower Vitamin D levels also had a significant impact on demographic markers. This study might provide a path and open research avenues in terms of Vitamin D association with disease severity. While the trends observed are consistent with existing knowledge, the study underscores the importance of continued research with larger cohorts and refined methodologies to unravel the intricacies of these relationships and inform more targeted clinical interventions.

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Conflict of Interest : None

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