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Research Article

VITAMIN D STATUS IN COVID-19 PATIENTS ADMITTED TO THE CRITICAL CARE UNIT OF AN EASTERN INDIA HOSPITAL

Avijit Saha[1*], Sangeeta Ghosh[2], Sandip Ghosh[3], Sanjay Vashistha[4], Shritama Aich[5], Tapas Kumar Sur[5]

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ABSTRACT: The COVID-19 patients experienced acute respiratory distress during their SARS-CoV-2 infections. The present observational study was undertaken to find out the possible association between vitamin D and COVID-19 clinical severity in a tertiary care hospital from November 2021 to February 2022. Patients admitted to the hospital's Critical Care Unit (CCU) with SARS-CoV-2 illness were selected for blood vitamin D estimations. Patients' conditions were evaluated clinically and correlated with serum vitamin D levels. Out of a total of 97 COVID-19 patients selected, 64.9% were male. Clinically, 41.3% of patients had a severe COVID-19 infection, compared to a moderate infection incidence of 58.7%. Among them, 19.6% and 42.2% were found to be vitamin D deficient and vitamin D insufficient, respectively. Serum vitamin D was significantly ($p<0.001$) reduced with age (≤ 60 yrs vs. ≥ 60 yrs) and with disease severe (moderate vs. severe). The insufficiency/deficiency of vitamin D was higher among the aged acute respiratory-infected COVID-19 patients. Therefore, vitamin D deficiency may be one of the main causes of severe illness in SARS-CoV-2 patients.

Key words: COVID-19, SARS-CoV-2, Vitamin D, Immunity, Acute respiratory distress.

INTRODUCTION

It is now well-documented that SARS-CoV-2 is a highly contagious virus and primarily causes upper respiratory tract infections in humans (Li *et al.* 2020). The structural spike protein of SARS-CoV-2 directly binds to Angiotensin-Converting-Enzyme-2 (ACE-2) receptors in alveolar cells in the lungs and leads to acute respiratory distress syndrome (ARDS) (Zemlin and Wiese 2020). In the recent COVID-19 pandemic, ARDS has been the prime cause of death (Xu *et al.* 2020). One of the key mechanisms for ARDS is the cytokine storm, which may trigger a vicious attack through the immune coordination of the body and cause multiple organ failure (Moore and June 2020, Jose and Manuel 2020).

While vitamin D insufficiency is a major public health dilemma worldwide in all age groups, it has been shown to offer protection against viral diseases such as the common cold, pneumonia, dengue, and hepatitis B and C in the recent past (Dankers *et al.* 2016). Studies on the epidemiology of disease have also shown a high correlation between seasonal variations in vitamin D, nutritional levels, and the frequency of respiratory infections (Grant 2008, Mandal and Pattanayak 2020). It has been hypothesized that vitamin D decreases the risks of infections by controlling physical barriers or enhancing cellular natural immunity or adaptive immunity and influencing metabolism (Cannell *et al.* 2008, Manna 2021). It interferes in the mainstream immune systems through macrophages, B and T lymphocytes, neutrophils, and dendritic cells (DiRosa *et al.* 2011). It is also speculated that vitamin D deficiency might increase the risk of respiratory tract infections including SARS-CoV-2 virus infection (Sooriyaarachchi *et al.* 2021, Dror *et al.* 2022). Furthermore, the role of vitamin D in reducing the cytokine storm on endothelium and alveolar membrane has also been proposed (Gombart *et al.* 2020). Hence, a deficiency in vitamin D might be associated with decreased immunity and increased susceptibility to infections, particularly in SARS-CoV-2 infection.

Recently, Heridari and his colleagues reported associations between the severity of COVID-19, serum vitamin D concentrations, and some inflammatory

[1] Department of Biochemistry, [2] Department of Microbiology, [3] Principal, [4] M.S.V.P, [5] Multidisciplinary Research Unit, R.G. Kar Medical College and Hospital, Kolkata, West Bengal, India.
** Corresponding author. e-mail: dravijitsaha81@gmail.com*

markers in pediatric COVID-19 patients in Iran (Heidari *et al.* 2022). But, Hernandez *et al*. (2021) did not find any relationship between vitamin D concentrations or vitamin deficiency and the severity of COVID-19 disease. A Meta-analysis reported SARS-CoV-2 infected patients with poor prognosis had a very low level of vitamin D as compared to those with favorable prognosis (Chiodini *et al*. 2021). Marik *et al*. (2020) hypothesized that hypovitaminosis D may contribute to the geographic variations in the documented case fatality rate of COVID-19. But, till now there are very scattered reports on the association of vitamin D with the severity of the COVID-19 disease. Therefore, the goal of the current investigation was to determine whether there was any association between serum vitamin D levels and the severity of the clinical symptoms experienced by COVID-19 patients.

MATERIALS AND METHODS

Subjects and methods

The present study was carried out at the Multidisciplinary Research Unit of a tertiary care hospital in Kolkata during the third wave of the COVID-19 pandemic in India, from November 2021 to February 2022. Ethical approval was given by the Institutional Ethics Committee (ECR/322/Inst/WB/2013/RKC/468). A total of 97 COVID-19 patients hospitalized with respiratory distress were included at random. The sample size was determined following a 1.96 confidence interval, 8% margin of error, and population proportion of 1 in 5 (*i.e*., 20%). After obtaining written informed consent, hospitalized adult patients (age 18 years and older) admitted to the CCU after having their COVID-19 status confirmed positive by RT-PCR and experiencing clinically diagnosed respiratory distress were recruited for the study. The study excluded individuals who had been diagnosed with any other serious illnesses such as cancer, HIV, stroke, trauma, cardiovascular disorders, chronic liver disease, renal failure, or any major surgeries. Patients who had just started taking vitamin D supplements were also excluded from the investigation.

The normal reference value of vitamin D was considered ≥ 30 ng/ml (Thacher and Clarke 2011). Patients were divided into three groups based on serum vitamin D levels: (a) normal ≥ 30 ng/ml, (b) insufficient 20-30 ng/ml, and (c) deficient, <20 ng/ml (Thacher and Clarke 2011). Further, patients who tested positive for COVID-19 were divided into three categories based on the disease severity following the guidelines of WHO (WHO 2020): (a) breathlessness absent in a mild case, (b) dyspnea and/or hypoxia present in a moderate case, (c) the respiratory rate between 24 and 30 breaths per minute, and the oxygen saturation between 90 and 94% in a severe case.

All enrolled COVID-19 patients' demographic data (age, sex, addiction history, co-morbidity history, etc.), clinical signs and symptoms, underlying medical problems, and hospitalization information were documented. Respiratory distress or SpO2 was assessed before giving them oxygen support. Within 24 hours of hospital admission, regular tests and vitamin D were performed. Serum vitamin D (25-hydroxycholecalciferol) was estimated using the chemiluminescence-based immunoassay analyzer ADVIA Centaur (Siemens, Germany) as per the manufacturer's instructions. The valid specificity range of vitamin D was 4.2-150 ng/ml. All measures were done within 2 hours of sample collection.

Statistical analysis

Descriptive analysis was performed where mean and standard deviation values were presented for all continuous variables, whereas number and percentage values were used for categorical variables. Data on clinical severity were compared to check the association between clinical severity, Vitamin D level, and age. Pearson correlation was used to examine the associations between categorical variables. The comparison between the two groups was done using a t-test. Data analyses were performed on SPSS version 20 (SPSS Inc., Chicago, USA). The significance level was set at 0.05.

RESULTS AND DISCUSSION

Out of 147 hospital-admitted COVID-19 patients during the time November 2021 to February 2022, only 97 patients were included in this study. Patients who were already taking vitamin D or had serious comorbidities mentioned above were excluded from the study. The selected patients (male 64.9%, female 35.1%) were distributed into different sub-sets like (a) age below 60 yrs (57.7%) and above 60 years (42.3%); (b) severity of COVID-19 denoted as mild (0%), moderate (58.7%) and severe (41.3%). The mean age of this study population was 59.74 yrs. The comorbidity conditions of the selected population were reported, 44.3% had hypertension, 35% had diabetes, and 18.5% had COPD (Table 1). Among the selected patients 19.6% were vitamin D deficient and 42.2% had inadequate levels of the vitamin. Table 2 depicted the comparison of serum vitamin D levels with age and severity of infection. The mean vitamin D level of patients with mild infection was 30.73 ng/ml for those under 60 years old and 29.24 ng/ml for those over 60 ($p<0.001$).

The vitamin D level of severely COVID-19-infected patients in the age group of ≤ 60 yrs was 24.86 ng/ml

and 23.37 ng/ml for patients ≥ 60 yrs ($p<0.001$). Table 2 indicated serum Vitamin D was significantly ($p<0.001$) reduced with age (≤ 60 yrs vs. ≥ 60 yrs) and severity of COVID-19 disease (moderate vs. severe). Fig. 1 highlights the relationship of vitamin D with the clinical severity of COVID-19 infections concerning oxygen saturation levels during hospitalization (A) and the ages of selected patients (B). Oxygen saturation level was linearly proportionate with vitamin D, while age was inversely proportionate with vitamin level. The Pearson correlation between vitamin D and the severity of COVID-19 diseases with age was demonstrated in Table 3. Moderate severity at age ≤ 60 yr was significant ($p<0.05$) with severe severity at age ≥ 60 yr. Significant correlations were reported between the severity of COVID-19 disease with serum vitamin D in patients.

A scientific report claims that half of the global population has vitamin D insufficiency causing serious health issues in both developing and developed countries (Amrein *et al.* 2020). Earlier, vitamin D deficiency was mainly focused on rickets or skeletal deformities in childhood and osteomalacia in adults. The vitamin enhances the absorption of calcium in the intestine and distal tubules of the kidney and regulates calcium metabolism (Holick 2007). Vitamin D deficiency has also been associated with multiple diseases including cardiac problems (Wang *et al.* 2008, Roy *et al.* 2015), diabetes (Mathieu *et al.* 2005), obesity (Vimaleswaran *et al.* 2013), depression (Nebhinani *et al.* 2017), and cancers (Lappe *et al.* 2007). In the present study, diabetes, hypertension, and chronic obstructive pulmonary disease (COPD) were the common co-morbid conditions with prevalence rates of 35%, 44.3%, and 18.5%, respectively. The clinical severity of COVID-19 and mortality were substantially correlated with diabetes and hypertension. Moreover, COVID-19 was frequently accompanied by aging and hypertension (Clark *et al.* 2021).

It has been reported that SARS-CoV-2 rapidly binds to ACE-2 receptors and suppresses ACE2 function in the lungs, triggering a pro-inflammatory cytokine storm that leads to multi-organ failure (Zemlin and Wiese 2020, Xu *et al.* 2020). A plausible role for vitamin D in preventing the cytokine storm by promoting the formation of ACE-2 has also been proposed by recent studies (Gombart *et al.* 2020, Jakovac 2020, Nimavat *et al.* 2021).

Vitamin D synthesis occurs under sun exposure, so a deficiency is anticipated if exposure is inadequate irrespective of availability (Pattanayak 2022). Multiple research studies have shown worse outcomes and greater infection rates of COVID-19 in countries with higher

Table 1. Characteristics of studied COVID-19 patients in CCU.

Parameter	Number
Number of patients	97
Male	63 (64.9%)
Female	34 (35.1%)
Age (mean ± SD)	59.74±12.83
Age <60 years	56 (57.7%)
Above 60 years	41 (42.3%)
Smoking habit	13 (13.4%)
Severity of COVID-19	
Mild	0
Moderate	57 (58.7%)
Severe	40 (41.3%)
Patients with underlying conditions	55 (56.7%)
COPD	18 (18.5%)
Diabetes	34 (35%)
Hypertension	43 (44.3%)
Vitamin D status	
Normal	37 (38.2%)
Insufficient	41 (42.2%)
Deficient	19 (19.6%)

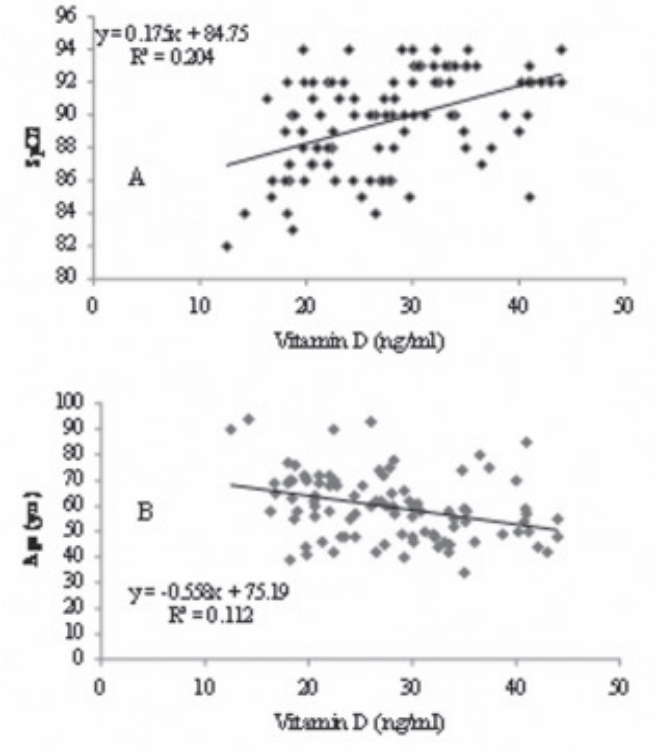

Fig. 1. Correlation between vitamin D and clinical severity of COVID-19 and age.

Table 2. Vitamin D levels in different age and severity of COVID-19 disease.

Age groups	Vitamin D level in COVID-19 disease severity		p-value (moderate vs. severe)	p-value (Moderate: ≤ 60 vs. ≥ 60 yrs)	p-value (Severe: ≤ 60 vs. ≥ 60 yrs)
	Moderate	Severe			
≤ 60 yr (n=56)	30.73±8.21 (n=38)	24.86±5.40 (n=18)	<0.001	<0.05	<0.05
≥ 60 yr (n=41)	29.24±5.20 (n=19)	23.37±7.75 (n=22)	<0.001		

*Results presented as mean ± SD, Vitamin D in ng/ml blood, t-test was used to determine the statistical significance between groups by using SPSS v20.

Table 3. Pearson's correlation between age, vitamin D and severity of COVID-19 disease.

Moderate Severity age ≤ 60yr	Moderate Severity age ≤ 60yr Vitamin D	Moderate Severity age ≥ 60yr	Moderate Severity age ≥ 60yr Vitamin D	Severe Severity age ≤ 60yr	Severe Severity age ≤ 60yr Vitamin D	Severe Severity age ≥ 60yr	Severe Severity age ≥ 60yr Vitamin D
1	0.301*	0.189	-0.083	-0.049	0.199	-0.031	-0.027
	1	0.242	0.081	-0.289	0.233	-0.216	0.-126
		1	-0.123	-0.057	-0.468	0.151	-0.240
			1	-0.153	-0.005	-0.228	0.067
				1	0.275	0.512*	0.094
					1	0.034	-0.030
						1	0.300

* Correlation is significant at the 0.05 level (2-tailed).

latitudes and lower vitamin D levels (Rhodes *et al.* 2020). This suggests vitamin D deficiency may have detrimental outcomes for COVID-19 patients. Insufficient vitamin D has also been related to high COVID-19 infection and fatality rates in European nations (Carpagnano *et al.* 2020). In India, the case fatality rate of the COVID-19 pandemic was less than 1.5%, and the mortality rate per 100,000 people was 37.5 as compared to 336.6 in the UK and 333.1 in the USA. (https://ourworldindata.org 2023).

In a systematic analysis of 28 observational studies (Jordan *et al.* 2022) incidence, severity, and mortality rate of COVID-19 infection were correlated with vitamin D levels. Two additional systematic studies come to the same conclusions (Ghasemian *et al.* 2020, Pereira *et al.* 2020). The present study found a substantial correlation between serum vitamin D levels and COVID-19 disease-related aging and acute respiratory distress. The overall prevalence of vitamin D deficiency was 19.6%. Furthermore, severe SARS-CoV-2 infected cases over the age of 60 were found to exhibit a 53.6% vitamin D insufficiency compared to 32.1% of cases under the age of 60. With advancing age, a strong association between vitamin D deficiency and acute respiratory distress of COVID-19 diseases was identified. Older persons had higher rates of SARS-CoV-2 infection with acute respiratory distress symptoms than the younger population. Singh and her co-workers (2021) also identified 58% vitamin D deficiency and 89% insufficiency among COVID-19 patients in cross-sectional research from India. Pinzon *et al.* (2020) reported 90% of COVID-19 patients in Indonesia had vitamin D insufficiency. According to Carpagnano (2020) COVID-19 mortality and severity were both allegedly correlated with vitamin D deficiency in several European nations. Moreover, inflammatory cytokines are involved in acute respiratory distress in COVID-19 disease and are linked to low levels of vitamin D, which can also manifest in these cases (Biesalski 2020). Thus, there is a greater likelihood that vitamin D supplementation could decrease the outcome of COVID-19 disease, including death in the vitamin D deficient population.

The investigation was conducted in only one center in Kolkata. The vitamin D level of normal people is also not well studied in India. In most of the localized studies reported a prevalence of 80%–90% deficiency among different population groups in India, taking very small number of samples (Aparna *et al.* 2018), but well studied data on vitamin D levels is not available for that region. The present study may need to be strengthened by a multi-center investigation.

CONCLUSION

According to the aforementioned observations, ARDS of the COVID-19 disease may be associated with vitamin D deficit or insufficiency. Additionally, patients with COVID-19 who are vitamin D deficient or insufficient should take a vitamin D supplement.

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