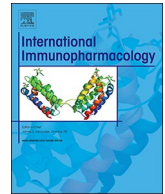




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Lower levels of vitamin D are associated with SARS-CoV-2 infection and mortality in the Indian population: An observational study

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ABSTRACT

Background: The role of vitamin D in the susceptibility and severity of various viral diseases has been well documented. Recently, some reports highlighted the possible importance of vitamin D in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although India receives adequate sunlight throughout the year, the majority of Indians are deficient in vitamin D levels. In the present study, we hypothesized that vitamin D deficiency would be associated with the SARS-CoV-2 infection rate and mortality in the Indian population.

Materials and methods: SARS-CoV-2 infection and mortality data were obtained from the Government of India's official website (accessed on 16th August 2020). Various literature databases like PubMed and Google Scholar were searched to find the mean of 25-hydroxyvitamin D [25(OH)D] levels in different states and union territories of India, Pearson correlation was carried out to investigate the possible link between mean 25(OH)D levels and SARS-CoV-2 infection and mortality per million of the population.

Results: An inverse correlation was observed between the mean level of 25(OH)D and SARS-CoV-2 infection rate ($r = -0.43$, $p = 0.02$) and mortality rate ($r = -0.42$, $p = 0.02$).

Conclusions: The present observational study revealed an association of vitamin D with SARS-CoV-2 infection and related mortality. Further studies are required to validate our observations.

1. Introduction

Novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China, in December 2019 [1] and has spread worldwide to 215 countries till date (<https://www.worldometers.info/coronavirus/>). On 11th March 2020, COVID-19 disease was declared a global pandemic by the World Health Organization (WHO) (<https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020>). India reported the first case of COVID-19 in a student who returned from Wuhan University China in Thrissur district of Kerala state on 30th January 2020 (<https://www.cnbcm.com/2020/01/30/india-confirms-first-case-of-the-coronavirus.html>). As on 16th August 2020, 2.59 million of SARS-CoV-2 infected cases has been reported in India, and a total of 50,431 death has been encountered. Maharashtra and Tamil Nadu states contributed the maximum number of infected cases and posed a high mortality of SARS-CoV-2 infected patients in India.

Individual immune systems, which are determined by several factors, play a major role in the susceptibility and pathogenesis of viral

diseases. The importance of vitamin D in the modulation of both innate and adaptive immune systems has been demonstrated [2]. In the skin, provitamin D₃ (7-dehydrocholesterol) is converted to previtamin D₃ after exposure to Ultraviolet-B (UVB) radiation. The previtamin D₃ is transported to the liver and hydroxylated at 25th carbon atom. The active metabolites 1,25(OH) vitamin D₃ is hydroxylated for the second time at C-1 at the kidneys [3]. Furthermore, 1-hydroxylation of vitamin D₃ is also reported in macrophages, monocytes, B and T lymphocytes [4]. Vitamin D deficiency has been associated with susceptibility to a wide range of viral infections, such as Human Immunodeficiency Virus, Influenza Virus, Epstein - Barr Virus, Hepatitis B, Human Respiratory Syncytial Virus [5]. However, the role of vitamin D in SARS-CoV-2 infection is not well understood. Some recent investigations have highlighted the beneficial role of vitamin D against SARS-CoV-2 infection and related clinical severity [6–13], by modulating the immune system [14]. In addition, a recent study in UK population reported the worst morbidity outcome in older patients with vitamin D deficiency [13].

Although India is close to the equator and receives a large amount of sunlight throughout the year, most of the Indian population (50–90%)

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is deficient in vitamin D [15]. In addition, differential levels of 25(OH)D have been observed in different geographical areas of India. Furthermore, unusual rates of SARS-CoV-2 infection and mortality have been observed in different states and Union territories of India. Based on these observations, we hypothesized that the mean 25(OH)D levels in the cohort would be correlated with the infection and mortality rate of COVID-19 in the Indian population.

2. Materials and methods

2.1. Vitamin D data

We investigated the correlation between vitamin D and COVID-19 disease in the Indian population to minimize the effect of various confounding factors such as ethnicity, latitude, health facility, etc. India is comprised of twenty-eight states and eight union territories (UTs) and estimated inhabitants of 1380 million equivalent to 17.7% of the total world population. Vitamin D levels in healthy adults were searched through PubMed and Google Scholar in the Indian population. All relevant articles were screened, and data such as the author's name, year of publication, number of healthy subjects enrolled for the analysis, mean \pm standard deviation or median, interquartile range of vitamin D were extracted.

2.2. COVID-19 data

SARS-CoV-2 data such as the number of cases, death figures, and the number of recovered persons were obtained from the official website of the Ministry of Health and Family Welfare, Govt. of India (www.mohfw.gov.in accessed on 16/08/2020). The population of each state and UTs were gathered from data of census performed in the year 2011 (<https://censusindia.gov.in/2011-common/censusdata2011.html>). SARS-CoV-2 cases per million and death per million were calculated by using the census-2011 data. Based on suggestions how to present numerical data appropriately [16], the infection rate per million were converted into multiples of ten. Death rate and mean vitamin D levels were represented in whole numbers.

2.3. Statistical analysis

Data on levels of 25(OH)D in the median and interquartile range were converted into mean \pm standard deviation format as described earlier [17]. The mean 25(OH)D levels in a state or UT with more than one report were pooled (<https://home.ubalt.edu/ntsbarsh/Business-stat/otherapplets/Pooled.htm>). The Pearson test performed a correlation of mean 25(OH)D levels with SARS-CoV-2 infection and mortality rate. All statistical analysis was carried out by GraphPad Prism 8.3.0. A p -value < 0.05 was considered as statistically significant.

3. Results

Out of 28 states and 8 UTs, mean 25(OH)D data was available for 23 states and 4 UTs (Andhra Pradesh, Assam, Chandigarh, Bihar, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Puducherry, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, Uttarakhand, West Bengal) (Table 1). However, there are eight states viz. Bihar, Goa, Himachal Pradesh, Jharkhand, Madhya Pradesh, Manipur, Meghalaya, Uttarakhand have only one report containing 25(OH)D levels in healthy adults (Table 1).

A possible correlation between the mean levels of 25(OH)D with SARS CoV-2 infection/million and mortality/million by Pearson correlation test and results are shown in Fig. 1. An inverse correlation was observed among mean 25(OH)D and SARS-CoV-2 infection rate/million in the Indian population ($r = -0.43$, $p = 0.02$) (Fig. 1A). Interestingly,

Table 1
SARS-CoV-2 cases, mortality rate and the mean 25(OH)D levels in different states and union territories of India.

State/ union territories	Case/ million	Death/ million	Total no. of healthy subjects	Mean 25(OH)D levels (ng/ml)	References
Andhra Pradesh	5530	50	1936	18	[32–36]
Assam	2390	6	768	26	[37–39]
Bihar	940	4	50	30	[40]
Chandigarh	1830	27	878	34	[41–46]
Delhi	8970	249	2919	15	[47–68]
Goa	7520	64	640	19	[69]
Gujarat	1270	45	160	30	[70,71]
Haryana	1800	20	26,460	21	[72–74]
Himachal Pradesh	560	3	105	25	[75]
Jammu and Kashmir	2240	42	841	28	[76–80]
Jharkhand	650	7	72	22	[81]
Karnataka	3460	61	263	18	[82,83]
Kerala	1240	4	206	25	[84–88]
Madhya Pradesh	600	15	50	37	[89]
Maharashtra	5100	173	4471	13	[90–97]
Manipur	1540	5	108	25	[98]
Meghalaya	410	2	109	23	[99]
Odisha	1300	8	413	21	[100–104]
Puducherry	5610	85	265	27	[105–109]
Punjab	1045	26	152	30	[110,111]
Rajasthan	860	12	175	15	[112,113]
Tamil Nadu	4520	76	991	21	[107,114–118]
Telangana	2560	19	715	20	[119–121]
Tripura	1890	14	224	25	[122,123]
Uttar Pradesh	730	12	389	17	[124–130]
Uttarakhand	1150	15	100	38	[131]
West Bengal	1210	25	2029	17	[132–138]

Note: SARS-CoV-2 data was accessed on 16.08.2020.

the mortality rate due to SARS-CoV-2 infection was also negatively correlated with vitamin D status of healthy Indian subjects ($r = -0.42$, $p = 0.02$) (Fig. 1B).

In the Indian population, higher prevalence of SARS-CoV-2 infections has been recorded in the younger age group, while the elderly have the highest death rate (<https://www.ha-asia.com/63-of-covid-19-deaths-in-india-are-of-60-age-group/>). Based on this observation, we hypothesized a potential link between SARS-CoV-2 infection or mortality rate in different age profile of the Indian population. We obtained age group data from census 2011 (<https://censusindia.gov.in/2011-common/censusdata2011.html>). Correlation analysis showed no substantial relationship between the mean vitamin D levels and the risk of infection in the younger population ($r = -0.16$, $p = 0.42$) or the rate of mortality in the older age group ($r = -0.24$, $p = 0.23$).

4. Discussion

This study is the first of its kind to investigate the association of vitamin D with SARS-CoV-2 infection and mortality rates in the Indian population. The current epidemiological investigation revealed a significant association of lower 25(OH)D with SARS-CoV-2 related deaths and susceptibility to infection.

Several studies have recently been performed in different populations to decipher the possible role of vitamin D in SARS-CoV-2 infection. A retrospective analysis of 3,48,598 UK biobank participants showed a higher chance of SARS-CoV-2 in subjects with lower levels of 25(OH)D [18]. However, the statistical significance levels had vanished after adjustment of other confounding factors. In addition, infected patients with SARS-CoV-2 had lower levels of 25(OH)D compared to those without a virus infection in Switzerland [5] and the Israeli

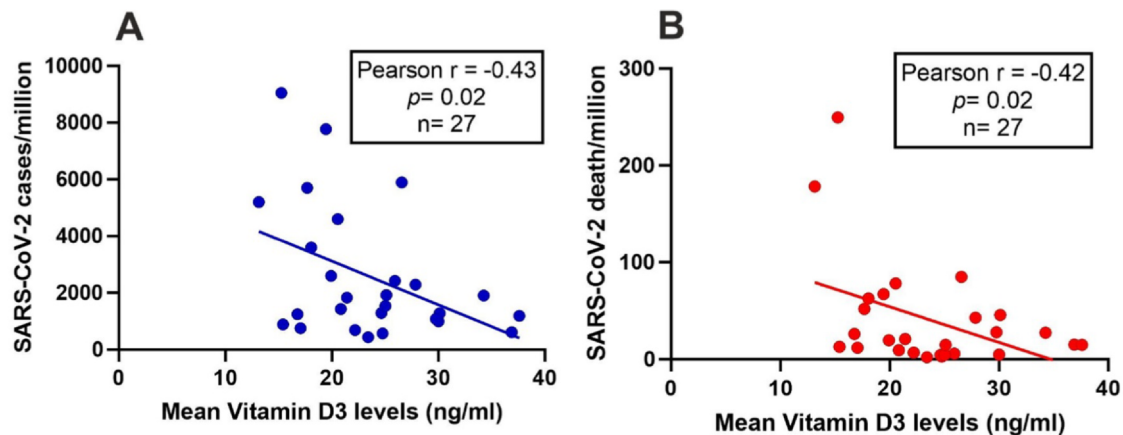


Fig. 1. Correlation of 25(OH)D with SARS-CoV-2 infection and death rate in the Indian population. Data on the number of subjects infected with SARS-CoV-2 and the number of death due to SARS-CoV-2, were obtained from the Government of India website (<https://www.mohfw.gov.in/>) (accessed on 16.08.2020). Mean 25(OH)D levels of different states and union territories populations were obtained from earlier published literature ($n = 27$). Pearson correlation analysis was performed to investigate the correlation of mean 25(OH)D levels with SARS-CoV-2 infection/million (A: $r = -0.43$, $p = 0.02$) and the mortality rate per million (B: $r = -0.42$, $p = 0.02$). A p -value of less than 0.05 was taken as significant.

population[7]. We observed a negative correlation in the Indian population between mean levels of 25(OH)D and the rate of infection with SARS-CoV-2 per million ($r = -0.43$, $p = 0.02$). Corroborating with the results of the present study, an analysis including data from 20 European continent countries also found a marginal inverse correlation between the mean 25(OH)D level and the rate of SARS-CoV-2 infection ($r = -0.44$, $p = 0.05$, estimated on 8th April 2020)[19]. Another independent analysis in the same population showed a stronger correlation [12], after reaching the post-COVID-19 infection peak (accessed on 12th May 2020). Lower levels of 25(OH)D have been linked to an increased risk of viral infections. Vitamin D induces the production of antiviral molecules such as cathelicidins and defensins, which decrease the rate of viral replication, decline the concentration of pro-inflammatory cytokines, and increase the production of anti-inflammatory molecules [20].

We examined the possible correlation in the Indian population between mean 25(OH)D level and COVID 19 mortality and found an inverse relationship ($r = -0.42$, $p = 0.02$). Recent reports including COVID-19 mortality data and mean 25(OH)D from 12 countries of European origin, also showed similar observations ($p = 0.04$). Similarly, another independent report involving records from 20 European countries revealed a marginal correlation ($r = -0.44$, $p = 0.05$, ased on 8th April 2020)[19]. However, after updating data on 12th May 2020, the relationship was disappeared[21]. Further, a recent report highlighted a higher chance of SARS-CoV-2 infected patients being admitted to an intensive care unit compared to those with insufficient or sufficient levels of vitamin D in the UK population[9]. The risk of hospitalization in SARS-CoV-2 infected subjects with lower levels of 25(OH)D was higher in the Israeli cohort [7]. A recent analysis revealed lower mortality rates of COVID-19 in the equatorial region compared to geographical areas away from the equatorial line[22], further reinforcing the importance of vitamin D against SARS-CoV-2 related death, as subjects residing at the equatorial line receive ample sunlight and possibly have higher levels of 25(OH)D. A meta-analysis of 61 independent studies including more than 10,000 COVID-19 cases demonstrated chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), cerebrovascular disease and coronary heart disease (CHD) as confounding factors for SARS-CoV-2 related death [23]. Severe vitamin D deficiencies have been associated with those confounding diseases such as CKD [24], COPD [25], cerebrovascular disease [26], and CHD [27].

Although several clinical trials have been registered to investigate the role of vitamin D supplementation in the treatment of SARS-CoV-2

patients (<https://clinicaltrials.gov/ct2/results?cond=Covid19&term=vitamin+d&cntry=&state=&city=&dist=>) majority of these trials are in the enrolment phase or have yet to be started. A recent study has shown that the administration of 1000 IU of vitamin D per day to four patients infected with SARS-CoV-2 leads to normalization of vitamin D levels, improved clinical condition, decreased oxygen requirements, reduced inflammatory markers, and shorter hospital stays [8]. In addition, another hospital-based randomised clinical study found that the use of calcifediol in infected SARS-CoV-2 subjects minimised the need for intensive care and reduced disease severity [28]. These observations further support the important role of vitamin D supplementation in the management of COVID-19 and necessitate completion of related clinical trials on an urgent basis to minimize the mortality rate worldwide.

Our study has several limitations, and those need to be disclosed. First, the investigation was not done in SARS-CoV-2 infected patients and healthy controls. Mean vitamin D levels and COVID-19 data of Indian states and union territories were obtained from the online database and earlier published reports. Second, seasonal fluctuations in mean vitamin D levels have been demonstrated [29,30], and the timing of the collection of the vitamin D data considered in this analysis is not known. Third, due to data unavailability, other confounding factors such as age, gender, comorbidity status, economic status, physicians, and nursing staff density have not been included in this analysis. Fourth, vitamin D exerts its cellular effect through vitamin D receptor (VDR) and transportation of vitamin D carried out through vitamin D binding protein (DBP)[31]. Various functional genetic variants believed to affect levels of VDR and DBP have not been included in the present study.

In conclusion, we observed an inverse correlation of mean 25(OH)D levels with SARS CoV-2 infection and mortality in the Indian population. The SARS-CoV-2 infection and mortality rate in India is increasing. For robust observations, it is advisable to re-analyze the correlation between 25(OH)D and COVID-19 once the infection rate has reached its peak. Besides, case-control studies, including a large number of subjects and randomized control trials in the Indian population, are required to validate our observations.

CRediT authorship contribution statement

Sunali Padhi: Investigation, Formal analysis. **Subham Suvankar:** Investigation, Formal analysis. **Venketesh K. Panda:** Investigation, Formal analysis. **Abhijit Pati:** Investigation, Formal analysis. **Aditya K. Panda:** Conceptualization, Supervision, Writing - original draft,

Writing - review & editing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2020.107001>.

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