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Original Research Article

Vitamin D in COVID-19

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) with clinical outcome ranging from asymptomatic to severe disease, and even death to some. It posed a terrifying challenge to healthcare system worldwide. Several observational and clinical trials has reported that, Vitamin D deficiency has contributed to acute respiratory distress syndrome. Case fatality rate increases with age and comorbidities, both of which are associated with decreased Vitamin D level. Therefore, this study is done to study the prevalence of 25(OH)Vitamin D in RT-PCR positive COVID-19 cases and RT-PCR negative controls.

Materials and Methods: This is a hospital based cross-sectional study conducted at Jawaharlal Nehru Institute of Medical Sciences, Manipur on 88 RT-PCR positive Covid-19 cases and 88 COVID-19 negative controls over a period of 2 years. Analysis of the sample was done by Liaison 25(OH) Vitamin D Total Chemiluminescence assay(CLIA).

Result: In this study, statistically significant (p-0.018) lower plasma 25(OH)Vitamin D level is seen in COVID-19 positive cases (median 28±20.47) when compared to Covid-19 negative controls(median 33.50±10.66). The number of 25(OH) Vitamin D deficient is higher in Covid-19 positive cases when compared to Covid-19 negative controls -46(52.3%) and 30(34%) respectively with a high statistically significant value (p-0.015).

Conclusion: COVID-19 positive cases have higher tendency to have suboptimal plasma 25(OH) Vitamin D level which may contribute to the high hospitalization risk in COVID-19 infection. This finding is important as it can identify population at risk, and contribute to interventions in reducing the risk of hospitalization associated with COVID-19 infection.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), with clinical outcomes ranging from asymptomatic to severe, including death. It continues to pose a terrifying challenge to healthcare system worldwide. In the hospital setting, therapeutic efforts to combat the severe complication are not always effective.[1] Thus, approximately 19.4% inpatients are transferred to ICU and 20.3% die from complications in patients infected with COVID 19.[2]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects pulmonary epithelial cells using the angiotensin converting enzyme-2 (ACE-2) receptor. SARS-CoV-2 also infects macrophages through ACE-2 receptors and activates them. There is sustained elevation of cytokines including interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF) alpha, resulting in type 2 pneumocyte apoptosis, and in some patients, it leads to acute respiratory distress syndrome (ARDS).[3] Sometimes there is an

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overwhelming expression of pro-inflammatory cytokines also known as 'cytokine storm' which is responsible for some of the serious manifestations of COVID-19 such as ARDS.[4]

Vitamin D plays an important immune regulatory role in suppression of the adaptive immune responses in respiratory epithelial cells via dampening T cell proliferation and the resultant shift from T helper type 1 (Th1) cells to T helper type 2 (Th2) during viral infection. Vitamin D can also influence T cell maturation and can divert the development of inflammatory T helper type 17 (Th17) cell mass towards anti-inflammatory regulatory T cell (T-reg cell).[5,6]

By virtue of its anti-inflammatory and immunomodulatory action several articles have documented the role of Vitamin D in reducing the risk of respiratory tract infection in COVID 19 context.

In this study, we investigated the prevalence of Vitamin D deficiency in COVID 19 patients admitted in our Covid dedicated ward a Tertiary care hospital and compared with COVID 19 negative patients admitted in General ward.

2. Materials and Methods

This is a hospital based cross-sectional study conducted in the Department of Biochemistry, Jawaharlal Nehru Institute of Medical Sciences, a tertiary care Hospital in Manipur. This study was done within a period of 2 years, starting from August 2020 to August 2022.

2.1. Inclusion criteria

Study population comprised of any Real time reverse transcription polymerase chain reaction (rRT-PCR) confirmed COVID-19 infection admitted in Covid ward, J.N. Institute of medical sciences, Porompat, Imphal. Age between 18- 70years including both male and female**.**

Age and sex matched Covid-19 negative participants were be recruited from Medicine ward and surgical ward, JNIMS Imphal.

2.2. Exclusion criteria

All those who did not give informed consent, patients with renal failure, patients with chronic liver disease, pregnant women, patients on Vitamin D supplements, patients suffering from acute illness like tuberculosis, sarcoidosis, hepatitis B and C, patients with history of malignancy, patients with history of osteoporosis, osteopenia under treatment, patients with history of cardiovascular disease under treatment were excluded from the study group.

2.3. Sample size

Based on prevalence of 81% of patients having Vitamin D serum level below 30ng/ml, with mean serum levels of 20.46 ± 11.6ng/ml[7] with a power of 90 and α value of 0.05(two sided) and effect size of 5.54 ± 11SD, the calculated sample size is found to be 88 in each arm.

2.4. Data collection

Subjects fulfilling the inclusion criteria were recruited from Dedicated Covid ward of JNIMS, Imphal. Age and sex matched COVID-19 negative participants were be recruited from Medicine ward and surgical ward of JNIMS, Imphal for controls. Blood samples were collected on the second or third day of admission. Baseline demographics, clinical history was retrieved from patient's medical record.

2.5. Classification of COVID-19 patient severity

Classification of the patients were done based on the guideline given by Ministry of Health and Family Welfare's criteria in India –

1. Mild: Without evidence of breathlessness or hypoxia (normal saturation).
2. Moderate: The presence of clinical features of dyspnea and or hypoxia, fever, cough, including SpO2<94% (range-90-94%) on room air, respiratory rate more or equal to 24 per minute.
3. Severe: Clinical signs of pneumonia plus one of the following; >30 breaths per minute, severe respiratory distress, SpO2 <90 on room air.[8]

Serum vitamin D:[9–16]

1. Normal/optimal - ≥30ng/ml
2. Insufficiency - 20-29ng/ml
3. Moderate deficiency - 10-19ng/ml
4. Severe deficiency - <10ng/ml.

2.6. Assay

Analysis of the sample was done by Liaison 25 OH vitamin D Total chemiluminescence immunoassay (CLIA).

2.7. Statistical analysis

Statistical analysis was done by descriptive statistics like mean, median, standard deviation etc. The categorical variables are described as frequencies and percentages. All data were assessed for normality using a Shapiro–Wilk test and the categorical data were compared using Mann–Whitney or Kruskal–Wallis statistical tests. Spearman's rank correlation was utilized to determine the continuous data. Statistical analyses were carried out using the SPSS software package, version 26. Two-sided $p< 0.05$ is considered as level of significance.

3. Result

A total of 176 patients (mean age 47.02 ± 12.09; male (n=99): female (n=77)) were recruited to the

study. Patients admitted in Covid ward has different complaints on presentation. 88(100%) of the subjects have generalised weakness, which is followed by fever 40(45.45%) and shortness of breath 35(39.7%). Diabetes mellitus is the commonest comorbidity observed, followed by Hypertension. The symptoms and comorbidities with their frequencies at the time of presentation/admission were given in Table 1. When 25(OH) vitamin D was categorised into sufficient (>30ng/ml) and insufficient (<29ng/ml), the number of low levels of 25(OH) Vitamin D was significantly higher in the COVID-19 cases when compared to the control group, 46(52.3%); 30(34%) respectively (p=0.015) shown in Table 2.

Table 2: Symptoms and their frequency in COVID 19 cases

Symptoms	**Number of cases (%)**
Generalised weakness	88 (100)
Fever	40(45.45)
Cough	25(28.4)
Shortness of breath	38(39.7)
Anosmia	17(19.32)
Loss of appetite	29(32.95)
Bodyache	20(22.72)
Comorbidities	
Hypertension	20(22.7)
Diabetes	25(28.4)
CVA	2(2.27)
CKD	1(1.14)
CAD	1(1.14)
Cancer	1(1.14)
Bronchial asthma	3(3.4)

Both the study groups were further categorised into 25(OH) Vitamin D sufficient(>30ng/ml), insufficient (29-20ng/ml), moderate deficient (11-19ng/ml) and severe deficient(<10ng/ml) and comparing them as categorical variables, the relationship between the study groups remained statistically significant(p=<0.001) shown in Table 3. However, no significant difference is seen in the level of 25(OH) Vitamin D between diabetic and non-diabetic patients (Figure 1).

The proportions of patients grouped by different thresholds of 25(OH)Vitamin D levels (≤ 10 ng/mL; 11–20 ng/mL, 20– 29 mg/mL and ≥30 ng/mL) for the mildly-symptomatic Covid19 patients, moderately symptomatic COVID 19 and severely symptomatic Covid19 is depicted in Figure 2.

As is evident, the prevalence of both vitamin D 25(OH)D < 10 ng/mL and 25(OH)D between 10 and 20 ng/mL was higher in moderate COVID-19 severely COVID-19 cases when compare to other groups. There is no significant difference in the level of 25(OH) Vitamin D across the age group (Figures 3 and 4).

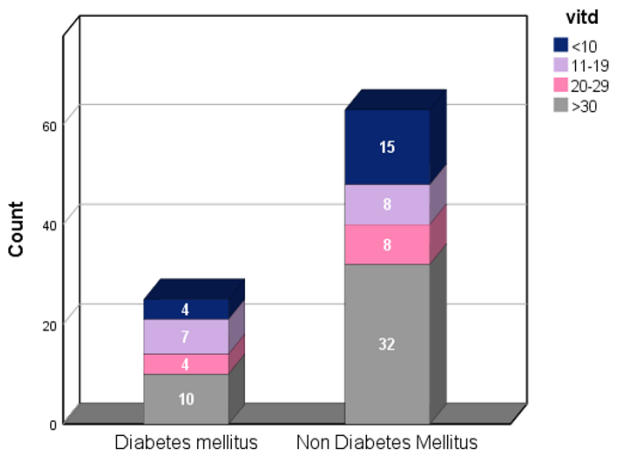

Fig. 1: Stacked bar showing diabetes mellitus by 25(OH) Vitamin D in COVID-19 cases

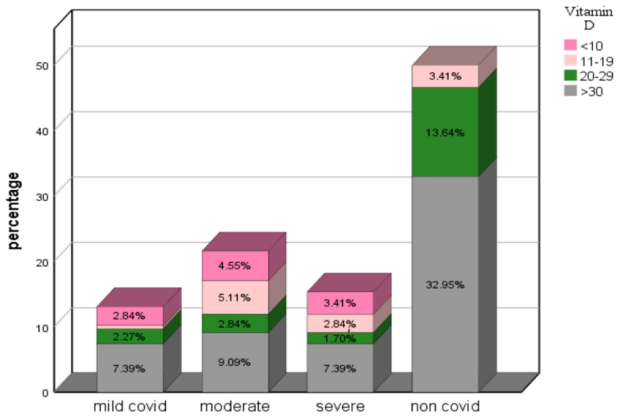

Fig. 2: Frequency distribution of 25(OH) Vitamin D in the study group

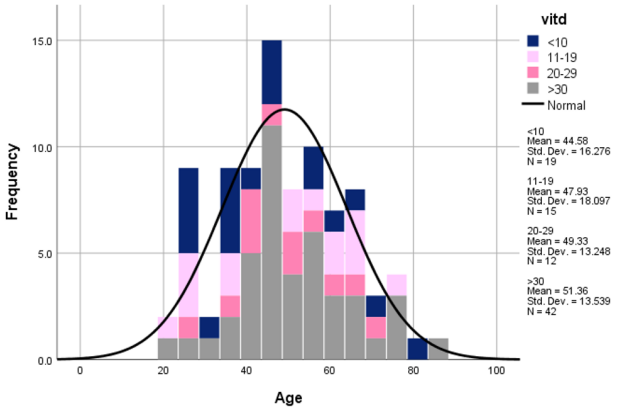

Fig. 3: Histogram of COVID-19 cases for 25(OH)D by age

Table 1: Frequency distribution of 25(OH) Vitamin D stratified by sufficient and insufficient in the study population

Controls			**Cases**			**p-value**
Vitamin D ng/ml	Number of controls	%	Vitamin D ng/ml	Number of cases	%	Value=5.93 df= 1
Insufficient (<29)	30	34	<29	46	52.3	p=0.015
Sufficient (>30)	58	66	>30	42	47.7	

Table 3: Different thresholds of 25(OH) Vitamin D level in the study group

Controls			**Cases**		
Vit D level	**Number of patients**	**%**	**Number of patients**	%	**p-value**
<10	0	0	19	21.6	
11-19	6	6.8	15	17.0	Value-29.417
20-29	24	27.3	12	13.6	df-3
≥30	58	65.6	42	47.7	p-<0.001
Total	88	100	88	100	

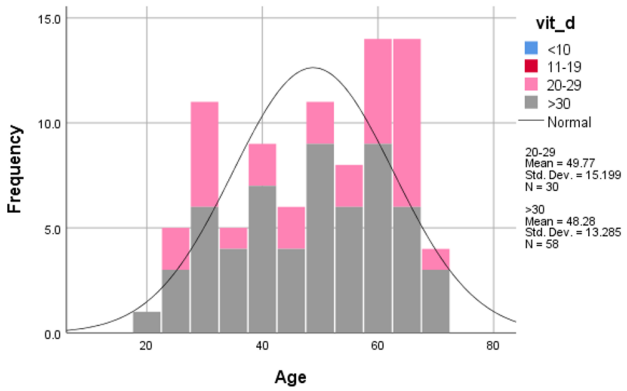

Fig. 4: Histogram of COVID-19 negative controls for 25(OH)D by age

4. Discussion

The main finding of this study is that there is significant difference in the level of 25(OH)D level between COVID 19 cases and the control group. The median 25(OH)Vitamin D ±SD levels among the control and case group are 33.50±10.66 and 28±20.47 respectively and has a high statistically significant value (p-0.018). The number of patients having insufficient 25(OH)Vitamin D level in control and case group was 30(34%) and 46(52.3%) respectively with a high statistical significance value (p =0.015). It is also observed that case group has higher number of patients with moderate and severe deficiency when compared to the control group, which is statistically significant(p=<0.001). This is in line with the finding by D'Avolio et al.[17] "the significantly lower 25(OH)D concentrations in the PCR-positive group could indicate that the risk of SARS-CoV-2 infection has a stronger relationship with the 25(OH)D concentration, rather than other respiratory tract infections." A study by Kun ye et al. also reported higher percentage of patients among severe/critical disease with 25(OH) Vitamin D deficiency compared to mild/moderate disease.[18] This report is in contrast with our findings in the present study, when comparison is done within COVID-19 cases, it is observed that, the percentage of 25(OH) Vitamin D deficiency is higher in the moderate disease but difference is statistically not significant(p=0.620). COVID 19 cases were further stratified into different age group and the 25(OH)Vitamin D level were compared. No significant difference was seen across the age group. This in agreement with the recent review and meta-analysis finding of Bassatne et al., he reported that, "Increasing the cut-off of low 25(OH)D levels to 30 ng/ml in our sensitivity analysis, revealed a significantly increased risk of mortality and testing positive for SARS-CoV-2 in patients with such levels. However, there were no associations with risk of disease severity and ARDS, and length of hospital stay."[19]

Diabetes mellitus being the most common comorbid condition seen in the present study, analysis is done on its association with 25(OH)vitamin D level in Covid-19 cases. When the median was compared between diabetic and non-diabetic group, its observed that, the difference between the two is not significant. Likewise, no significant difference with other comorbidities like hypertension, CVA which may be due to small sample size.

5. Limitation of the Study

As a single-centre study at a tertiary hospital, we cannot generalise our results to other settings. Selection of participants were limited to those cases where medical information can be retrieved from the medical records which require a second-hand interpretation and may not represent the full clinical picture. Despite achieving significant results in several outcomes, we acknowledge the risk of a type 2 error occurring with our experimental sample size.

6. Conclusion

In the present study we found that, suboptimal plasma 25(OH) vitamin D levels may be a potential risk factor for COVID-19 infection, particularly, for the high hospitalization risks, independent of demographic characteristics and medical conditions. This finding is important, since it could guide healthcare systems in identifying populations at risk, and contribute to interventions aimed to reduce the risk hospitalization due to COVID-19 infection.

In many countries people are taking a diet which is deficient and lacks micronutrients. A healthy diet including micronutrients more than RDA can boost our immune system and makes it stronger against viral infections. Those who develop COVID-19, should be screened for 25(OH) Vitamin D levels and if found insufficient/deficient, proper treatment should be given.

More studies are required to assess the effects of Vitamin D3 supplementation in reducing the risk of hospitalizations due to COVID-19 infection.

7. Source of Funding

None.

8. Conflict of Interest

None declared.

References

1. Bianconi V, Violi F, Fallarino F, Pignatelli P, Sahebkar A, Pirro M. Is acetylsalicylic acid a safe and potentially useful choice for adult patients with COVID-19? *Drugs*. 2020;80(14):1383–96.
2. Rosenthal N, Cao Z, Gundrum J, Sianis J, Safo S. Risk factors associated with in-hospital mortality in a U.S. national sample of patients with COVID-19. *JAMA Netw Open*. 2020;3(12):e2029058. doi:10.1001/jamanetworkopen.2020.29058.
3. Mcgonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev*. 2020;19(6):102537. doi:10.1016/j.autrev.2020.102537.
4. Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med*. 2020;8(6):46–7.
5. Azrielant S, Shoenfeld Y. Vitamin D and the Immune System. *Isr Med Assoc J*. 2017;19(8):510–1.
6. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. *J Pharmacol Exp Ther*. 2008;324(1):23–33.
7. Kara M, Ekiz T, Ricci V, Kara Ö, Chang KV, Özçakar L. Scientific Strabismus' or two related pandemics: coronavirus disease and vitamin D deficiency. *Br J Nutr*. 2020;124(7):736–41.
8. Does serum vitamin D level affect COVID-19 infection and its severity? -A case-control study. Available from: https://www.mohfw.gov.in/pdf/ClinicalManagementProtocolforCOVID19.
9. Alipio M. Vitamin D Supplementation Could Possibly Improve Clinical Outcomes of Patients Infected with Coronavirus-2019 (COVID-2019). *SSRN Electron J*. 2020;doi:10.2139/ssrn.3571484.
10. Raharusun P, Priambada S, Budiarti C, Agung E, Budi C. Patterns of COVID-19 Mortality and Vitamin D: An Indonesian Study; 2020. Available from: https://emerginnova.com/patterns-of-covid19-mortality-and-vitamin-d-an-indonesian-study/.
11. Laird E, Rhodes J, Kenny RA. Vitamin D and Inflammation - Potential Implications for Severity of Covid-19. *Ir Med J*. 2020;113(5):81.
12. Hribar CA, Cobbold PH, Church FC. Potential role of vitamin D in the Elderly to resist COVID-19 and to slow progression of Parkinson's disease. *Brain Sci*. 2020;10(5):284. doi:10.3390/brainsci10050284.
13. Fabbri A, Infante M, Ricordi C. Editorial - Vitamin D status: a key modulator of innate immunity and natural defense from acute viral respiratory infections. *Eur Rev Med Pharmacol Sci*. 2020;24(7):4048–52.
14. Boucher BJ. The problems of vitamin D insufficiency in older people. *Aging Dis*. 2012;3(4):313–29.
15. Crane-Godreau MA, Clem KJ, Payne P, Fiering S. Vitamin D deficiency and air pollution exacerbate COVID-19 through suppression of antiviral peptide LL37. *Front Public Health*. 2020;8:232.
16. Ghavideldarestani M, Honardoost M, Khamseh ME. Role of vitamin D in pathogenesis and severity of COVID-19 infection. *Preprint*. 2020;doi:10.20944/preprints202004. 0355.v1.
17. D'Avolio A, Avataneo V, Manca A, Cusato J, DeNicolò A, Lucchini R, et al. 25-Hydroxyvitamin D Concentrations Are Lower in Patients with Positive PCR for SARS-CoV-2. *Nutrients*. 2020;12(5):1359.
18. Ye K, Tang F, Liao X, Shaw BA, Deng M, Huang G, et al. Does serum vitamin D level affect COVID-19 infection and its severity? -A case-control study. *J Am Coll Nutr*. 2020;13:1–8.
19. Bassatne A, Basbous M, Chakhtoura M, Zein OE, Rahme M, Fuleihan GE. The link between COVID-19 and VItamin D (VIVID): A systematic review and meta-analysis. *Metabolism*. 2021;119:154753. doi:10.1016/j.metabol.2021.154753.

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