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Relationship between Vitamin D Insufficient, Vitamin D Deficient Pakistani population and COVID-19

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***Abstract*---**The people with less Vitamin D have enlarged probability for acute respiratory tract infections. There is substantial uncertainty about the link between "25(OH)D" ratio and the prevalence of "COVID-19". With the major endpoints of death, duration of staying in hospital, cannulation and rate of recurrence of COV-19 indicators, this study was done to evaluate the quantity of Vit-D in Corona patients in Jinnah post graduate medical Centre Krachi, Pakistan, and to link "vitamin D" insufficiency and shortage with these events. Eighty patients in total had their "25(OH)D" status assessed throughout the duration of their COVID-19 disease. Deficient (9 nanogram/milliliter), inadequate (5-35 ng/mL), or appropriate (> 25 ng/mL) 25-hydroxyvitamin D levels were determined. Patients in

ICU (P = 0.035), protruding oxygen supply (P = 0.03), tenacious eosinophilic pneumonia, and decease were all substantially correlated with “Vit-D” insufficiency in univariate Cox regression. After adjusting for confounding variables, only death (P = 0.039) and persistent pulmonary infiltrates (probability = 0.008) were statistically substantial on multivariate Cox regression. Moreover, affected population having 25(OH)D concentrations 9.9 ng/mL showed a better likelihood of surviving than others. Populations lacking in 25-hydroxyvitamin D experienced lower results and a long duration of hospital stay.

***Keywords*---**Vitamin D Deficient, COVID-19, Relationship.

Introduction

The COVID-19 coronavirus illness contagion has swiftly proliferated globally, affecting practically every nation. 100 million and above, COVID-19 infections and deaths were stated as of late, representing astounding numbers. The COVID-19 outbreak has become a major public health emergency that is putting the world's healthcare systems to the test. It has been determined that underlying medical issues are related to both the severity of the cases and the morbidity (Jayawardena, Jeyakumar, Francis, & Misra, 2021). Comorbid conditions like diabetes and coronary artery disease, as well as stress and hyperactive immune cell populations and activity, can exacerbate the extent of the illness (Erener, 2020). Furthermore, obesity as a probable risk factor for an underlying chronic condition was studied. Results revealed that overweightness was pointedly linked to the overall affected people and COVID-19 transience in a million people (Jayawardena, Jeyakumar, Misra, Hills, & Ranasinghe, 2020). As the major region, Asia is home to an estimated 60 percent of total of the world's population, which is rapidly approaching 4.7 billion people. Economic, racial, and underlying health disparities may all work together to amplify the negative effects of COVID-19 (Jayawardena et al., 2021). Immunological disruption is the main characteristic of COVID-19, thus reestablishing immunological stability and avoiding the over production of cytokines due to inflammation could be an approach to fight the disease (Ma, Zhou, Xu, Ma, & Wang, 2020). The immune system depends on the ranges of minor nutriments, including vitamins A and D. these are particularly helpful in populations with vitamin deficiencies (Jayawardena, Sooriyaarachchi, Chourdakis, Jeewandara, & Ranasinghe, 2020). A number of studies have hypothesised a connection between COVID-19 vulnerability and the deficiency level of this vitamin (Dissanayake et al., 2022; Grant et al., 2020; Rhodes, Subramanian, Laird, & Kenny, 2020). The most important steroidal hormone is Vit-D which is either exogenously obtained from diet or other medicinal supplements or created biochemically via ultraviolet visible rays activity on the skin (Ali, 2020). It has been demonstrated that this vitamin is essential for treating acute infections of the airways (Martineau et al., 2017). In particular, vitamin D regulates innate and cell mediated immunity, cytokine release and inflammation, and may lower the risk of infections via a number of pathways (Tay, Poh, Rénia, MacAry, & Ng, 2020). The normal people, the people with severe COVID-19, and those with nonfatal COVID-19 are compared, Corona

affected persons showed reduced “25(OH)D” concentrations (Dissanayake et al., 2022). Less defensive functioning of body and higher vulnerability to infections are linked to vitamin D deficiency. The mechanisms of Vit-D are involved in the natural cell induced immunity, the physiochemical barrier, and immunity persuaded via adaptation. Junctions(tight, adherens, and gap)require proteins for normal functioning. The production of these proteins are controlled which get affected by microorganisms, leads to strengthen the physical barriers related to immune system (Bae & Kim, 2020). Another vitamin D form with a well-known antioxidant effect is 25-hydroxyvitamin D. Despite conflicting results, there is reason to believe that this viral infection and ergosterol levels are related. This relationship merits more research (Asghar et al., 2022). The determination of this research was to ascertain the connection between “COVID-19” and a lack of vitamin D in the Pakistani population.

Methods

At Jinnah Post Graduate Medical Centre(JPMC) Karachi, comprehensive research was done. 91 individuals' vitamin D level was evaluated over the duration of their COVID-19 illness. When these patients were admitted, the 25(OH)D was determined by obtaining a sample(serum) volume of 9-14 ml for automated kit method using immunoassay based on enzyme. For this experiment, serum was drawn into standard sample tubes or tubes containing EDTA. At 20–25 °C, 25-hydroxyvitamin D is constant for 7.9 hours. Precipitate-containing samples were centrifuged for two hours before testing. 3.0 ng/ml is the minimum detection limit. PCR technique was used to determine whether a nasopharyngeal or oropharyngeal swab contained COVID-19 infection (PCR). Vit-D levels in sera less than 9.9 ng/mL were well-thought-out as deficient, between 9 and 29 ng/mL as insufficient, and over 30 ng/mL as adequate. Based on “25(OH)D” levels, data was divided into 2 categories that were lower or greater than 10 ng/mL to analyze the impact of “25(OH)D” concentration in connection with the clinical characteristics of the disease. The primary outcomes were transience, duration of staying in hospital, cannulation, and incidence of indications (Cov-19). 2ndry conclusions included comorbid relationships, pulmonic intrudes, and in-hospital proceedings. The reviewed documents and information were stored in automated therapeutic archives of the hospital. They were used to determine/extract, gender, age, body mass index (BMI), and consequence information. According to guidelines provided by the World Health Organization, BMI for people at high risk was classified as being below or above 26 kg/m2. To investigate the effects of different effect converters on 25(OH)D status, simple linear regression was used. Cox regression (Univariate and multivariate) analysis was done to calculate the risk proportion for substantial variable quantity. To assess the confidence intermission of the Vit-D scarce category with power-driven breathing, the resolution of **eosinophilic pneumonia** on X-ray of the chest, and subsistence throughout the clinical stay, Kaplan Meier curves were built. The endurance circulations among different groups were contrasted using a <log-rank test>, and the probability value of ≤0.05 or lower was supposed to be statistically important. The data is analyzed through excel and descriptive statistics were used to report the variables.

Eligibility criteria

Adult patients with an infection from SARS, MERS, or COVID-19 make up the population of interest. In observational research, the exposure is vitamin D status, while in clinical trials, any form or amount of vitamin D is the treatment.

Data analysis

Utilizing SPSS, a statistical analysis was carried out. The Kolmogorov-Smirnov trial was done for analyzing the statistics familiarity. Pearson correlation coefficients were used to examine the link between the followings, a) independent variables, b) prevalence of VDD and c) mean VD level. The other factors include the d) dependent variables, e) total COVID-19 infections and f) mortalities. Multiple linear regression was used as a predictive analysis to ascertain the relationship of SARS-COV2 invasion and the death rate. The relation of COVID-19 and Vit-D deficiency and mean level of Vit-D. The other factors include age of population and frequency of overweightness. Scattered diagrams were used to visually show all the results, and Microsoft Excel regression lines were used to assess them.

Results

The majority of the population under investigation had insufficient <vitamin D> levels. All patients exhibited average “25(OH)D” concentrations of 15.8 ng/mL, with 8.5% having inadequate levels, 56.5 having deficient proportions, and 22.6% having acceptable quantities. 68.1% of the participants in the study were men (n=60). The range of their age was 53 years on average. The cases were handled in intensive care units for the remaining cases and isolation wards for 69.2% of the cases (ICUs). Table 01 shows that 20.9% of patients died while they were hospitalized. Vitamin D deficient individuals were more susceptible to be mortal, aggressive aeration, ICU admission (P = 0.039), and persistent pulmonary infiltrates (P 0.001). First (P = 0.043) and last (P = 0.008) above mentioned factors were persisted statistically significant on multivariate cox regression after adjusting for confounding factors. According to table 02, almost 80 % population had fever and about 75% people have cough.

The low level of 25(OH)D (9.8 nanogram per milliliter) in patients is associated with the increase in incidence of symptoms including cough with sputum, hypernea/shortness of breath fatigue, Sickness, looseness of the bowels, anosmia and nasal mobbing. In addition, the patients with longer hospital stays were more likely to require intubation.

Determination of serum 25(OH)D levels

Intravenous puncture was done to collect venous blood as a sample (2.5 ml) from each participant, in yellow gel tubes with tops. Enzyme-linked immunoassay (ELISA) kit was utilized to measure the amounts of serum Drisdol. The Parathyroid Hormone (PTH) levels were utilized as the additional factor to define VDD. Serum 25(OH)D values below 9 ng/ml were considered to be less frequency of vitamin D.

Table 01. Patient demographics and clinical traits in relation to mean 25-hydroxyvitamin D levels. ICU stands for an intensive care unit. BMI is body mass index. Whitney Mann P values are computed using the U-test.

Variables	**%age**	**25-H-Vit D level**	**P-Value**
Age (years)			
30-50	**48%**	**25±7**	**0.27**
50-70	**57%**	**23±6**	
Gender			
Male	**71%**	**25±9**	**0.23**
Female	**34%**	**22±5**	
BMI			
Greater than 30	**55%**	**25±3**	**0.26**
Less than 30	**50%**	**23±5**	
Stay in seclusion	**72%**	**26±8**	**0.007**
Stay in ICU	**33%**	**19±5**	
Invasive ventilation	**15%**	**14±4**	**0.003**
Non-invasive ventialtion	**89%**	**25±5**	
Improved	**82%**	**26±5**	**0.001**
Death	**23%**	**14±3**	
Pulmonary infiltrates (✓)	**76%**	**26±9**	**0.003**
Pulmonary infiltrates (Ø)	**29%**	**18±10**	

Lack of calciferol Increases Corona virus Risk and Severity

The indication that Vit-D level impacts the SARS-COV incidence arises mostly after databased and biological research, which is primarily because to the recentness and uniqueness of this virus. People with less than 4.9 ng/mL “25(OH)D” concentration received 290,000 IU of vitamin D3 and then 1500 IU each day. Patients who had <25(OH)D> levels in 4.9 and 9.9 ng/mL received 100,000 IU of vitamin D, trailed by 700 IU every day. Patients who had cholecalciferol levels between 9.9 and 15.9 ng/mL received 95,000 and then 700 IU everyday. While individuals with “25(OH)D” more than 25 ng/mL did not get vitamin D, those with levels between 15.6 and 29.5 ng/mL received 750 IU/d. Baseline 25(OH)D concentrations were not related to death (average) probably as a result.

Table 02. In a linear regression of 25-hydroxyvitamin D status with research parameters, vitamin D levels are the dependent variable.

Variables	**95% Confidence interval Upper lower**	**Standard error (SE)**	***P* value**	**Unstandardized coefficients beta**
Ward/ICU	-13.5 -5.6	3.2	0.003	-5.6
Recoveries vs. death	-10.4 -4.9	2.2	0.002	-4.6
Resolving vs. persistence of pulmonary infiltrates	-15 -5.8	2.4	0.001	-8.4
Noninvasive vs. invasive ventilation	-20.3 -7.5	3.9	0.001	010.2

25-hydroxyvitamin D levels of less than 10 ng/mL as the outcome variable in a multivariable Cox regression						
		Univariate analysis		**Multivariate analysis**		
Variables	**Risk ratio**	**P-value**	**95% CI**	**Hazard ratio**	**P-value**	95% CI
Isolation Ward ICU	2.1 2.2	0.003	1-2.1	2.1 2.3	0.43	0-4.82
Recoveries Death	2.3 3.8	0.001	1-4.8	2.3 3.1	0.0039	1-4.2
Resolving persistence of pulmonary infiltrates	2 (no) 8 (yes)	0.001	2.5-21.4	2.3 7.7	0.008	1.5-27.5
Noninvasive invasive ventilation	2.1 3.5	0.03	2.1-7.6	2.0 2.2	0.7-1.10	0.03

Vitamin D and COVID-10 Treatment

With high-dose treatment, biomarkers of inflammation were significantly decreased: CRP decreased from 30 to 1.5 milligram/deciliter and 16 to 6 mg/dL, respectively, likened with 12 to 22 mg/dL and 20 to 17 mg/dL for treatment with less quantity. IL-6 decreased from 13 and 9 pg/mL to 4.5 pg/mL for high-dose treatment, compared with 4.5 and 5.5 pg/mL to 4 and 7 pg ICU care was not necessary for 49 of the patients receiving calcifediol treatment, but it was for 13 of the 26 patients who weren't. moreover, two of the ICU effected persons that were

admitted passed away. The hostile ventilation, resolution of lung penetrates, and existence over a month hospital stay [Kaplan Meier curves] of 25(OH)D insufficiency. For the resolution of pulmonary infiltrates, Patients with ergosterol deficiencies (9.5 ng/mL) had a higher likelihood of demanding machine-driven breathing compared to those having this hormone greater than 9.9 ng/mL}. Under the examination period of staying in hospital for 27 days, early power-driven aeriation was given to the inadequate group. Age, Vit-D status, and their relationships to mortality, ICU stays, and the need for ventilating system as a result of this infection are all related. Scatter graphs were formulated for exhibition of this hormone with respect to age. Figure 04 illustrates a mildly negative association, or the possibility that <25(OH)D status> may decline to some extent as age rises (R2 = 0.024). ICU stays are pointedly longer in victims whose Vit-D levels are below 9 ng/mL. Additionally, the peak in inpatient impermanence is highly noticeable at lesser extent of “25(OH)D”. The majority of affected persons recovered with noninvasive ventilation, while some patients with insufficient <25(OH)D> need invasive ventilation.

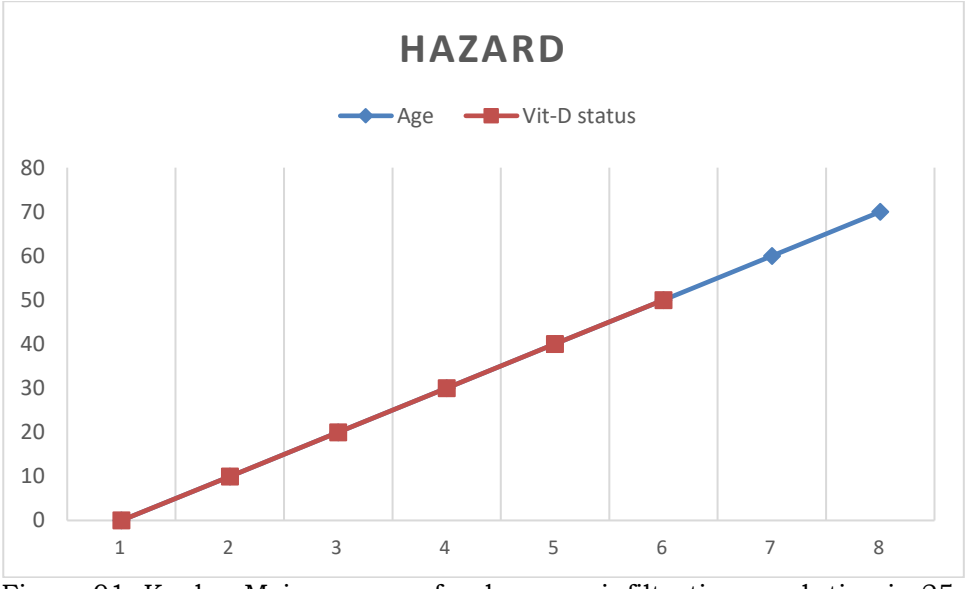

Figure 01. Kaplan Meier curves of pulmonary infiltration resolution in 25-hydroxyvitamin D insufficiency

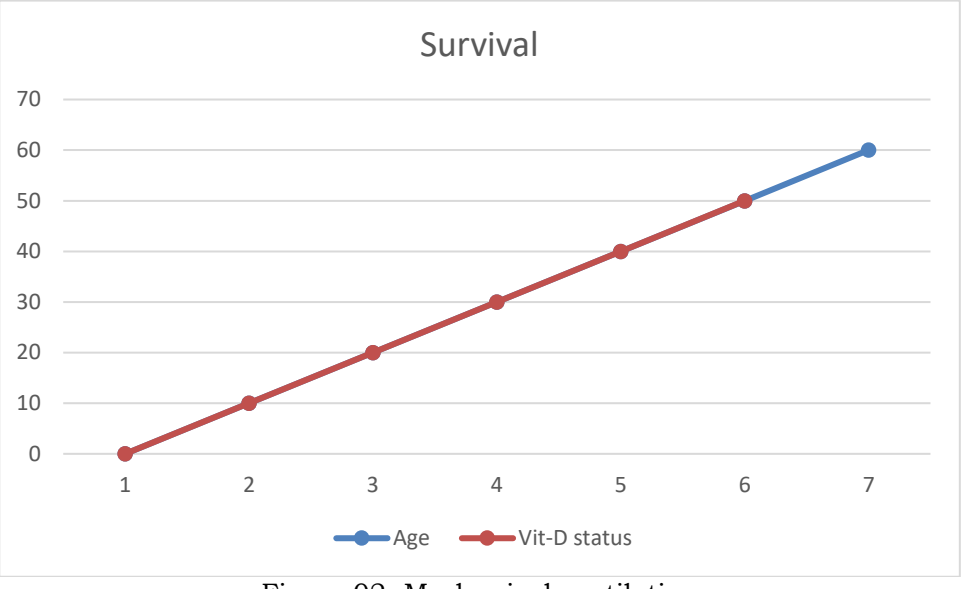

Figure 02. Mechanical ventilation

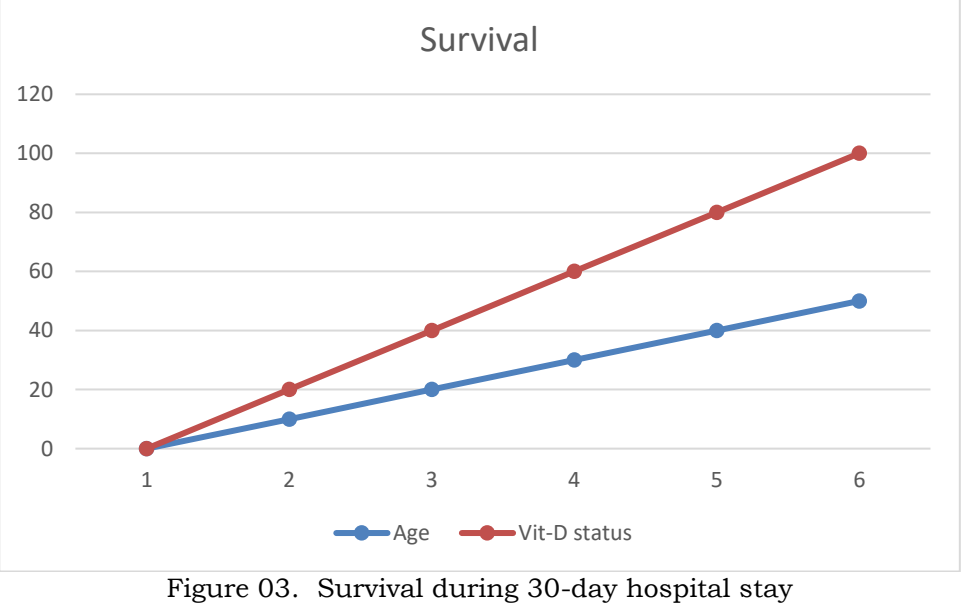

Figure 03. Survival during 30-day hospital stay

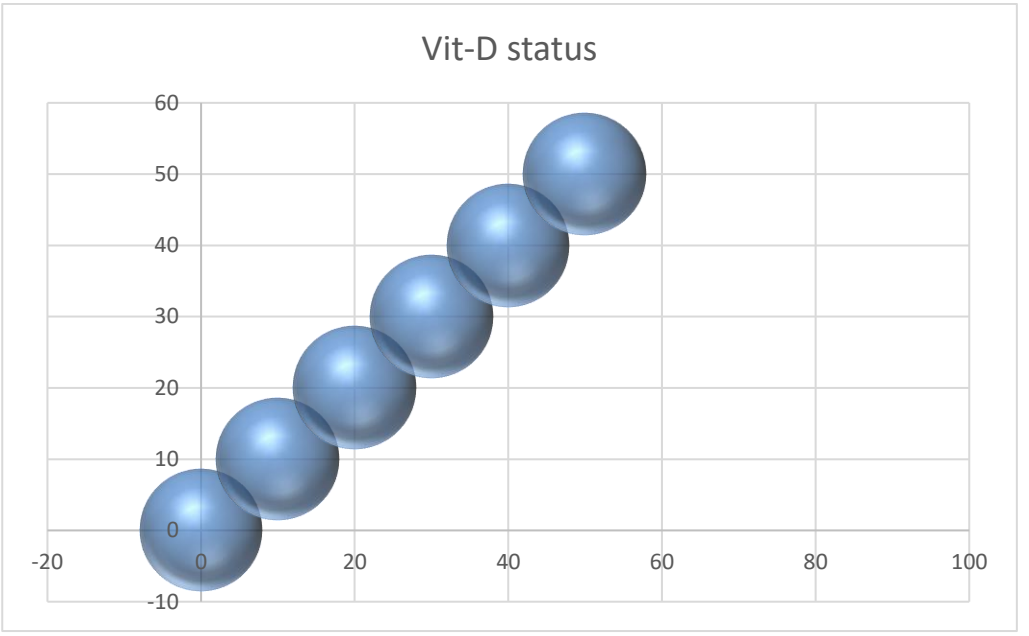

Figure 04. Age related Vit-D status

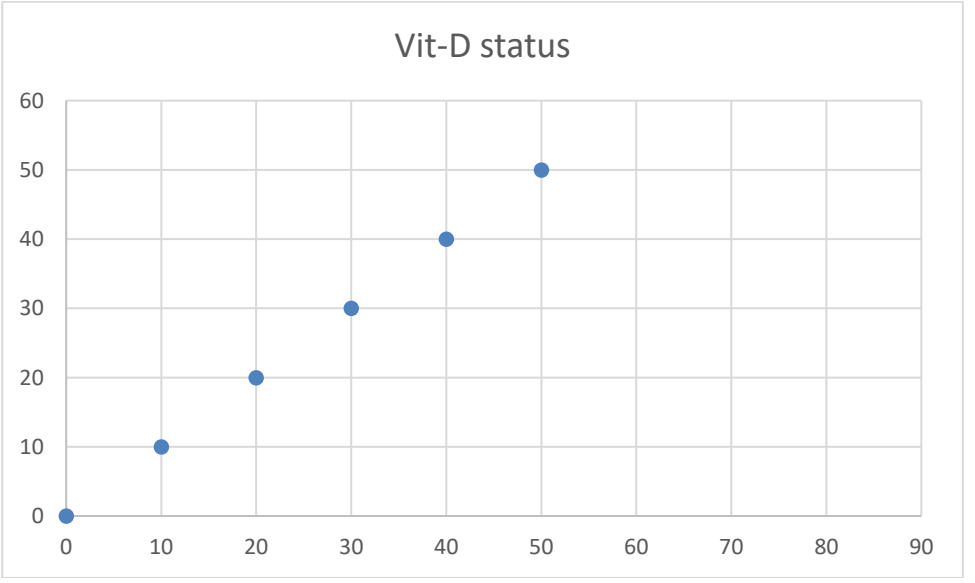

Figure 05. Clinical outcomes after normalizing Vit-D level

Discussion

Based on research, [25(OH)D] is essential for controlling the RAS(renin-angiotensin system). Already formulated data showed that, severe acute respiratory syndrome coronavirus-2 uses the "Angiotensin" converting enzyme (ACE-II) receptors to gain internal access to the cell. For controlling the virus, "25(OH)D" controls a variety of immunological responses, including reducing

SARS-CoV-2 entrance and replication and reducing hyperinflammation by elevating levels of anti-inflammatory cytokines (Kumar, Rathi, Haq, Wimalawansa, & Sharma, 2021). Vit-D aids in the production of antimicrobial peptides of proteins. Chemotaxis is one of the main functions of AMP, or the migration of cells in response to a biochemical inducement. The innate immune system is activated, angiogenesis is impacted, antiendotoxin activity is increased. Lipopolysaccharide, or LMS (lipopolysaccharide)(Crane-Godreau, Clem, Payne, & Fiering, 2020). This study was based on research at a solitary institution, the limited sample size is one of the study's main limitations. Convenience sampling-related collider bias is a possibility that cannot be completely ruled out. This limits the generalizability and power of our findings. The presence of additional determinants, which may explain variations in 25(OH)D status and outcomes and have an impact on the reliability of the results, is another significant limitation of this study. For instance, a lower level of vitamin D may result from fewer outside activities owing to illness. Similar to how seasonal change can affect levels, age, pregnancy, and thyroid function can too. The ability to detect a significant difference will be further diminished by a small sample size. However, our findings imply that 25(OH)D deficit may be associated with exacerbated symptoms, invasive procedures, and intensive care unit stays, so, lowering mortality. Ergosterol levels that are sufficient were also linked to promoting persistence and accelerating retrieval. Swelling may thus dysregulate vitamin D metabolism (Waldron et al., 2013). A prothrombotic condition, which is linked to both COVID-19 infection and vitamin D insufficiency, is another related connection. It is advisable to add vitamin D to the treatment plan if Vit-D is operative in lessening the sternness of {COVID-19} in order to lessen the pandemic's effects (Weir, Thenappan, Bhargava, & Chen, 2020). Additionally, serious disease consequences in the old age sample of population are highly deficient in Vitamin D, when COVID-19 positive is linked to low “25(OH) D” levels. Lower ranges of “25(OH)D” were found in died patients in comparison with those who had been released, and those with sufficient amounts of 25(OH)D had less lung involvement on computed tomography (CT) scans.

Conclusion

Rising data suggests that lower hazard and brutality of COVID-19 are correlated with higher “25(OH)D” concentrations in blood. A number of mechanisms, including preserving healthy epithelial layers, lowering viral survival and replication, lowering the generation of pro-inflammatory cytokines, and raising ACE2 concentrations, may be at play in this process. Further research is required to determine the pathways through which vitamin D can regulate the prevalence of “COVID-19”. Those who were deficient in 25-hydroxyvitamin D had prolonged hospitalisation, shorter survival rates, and far worse results. The clinical features of the deficient group also differed substantially. Vitamin D may benefit the immune system, as evidenced by the lower incidence of invasive procedures, complications, and fatalities in patients with adequate 25(OH)D levels. If the individuals having this life-threatening viral disease and lower range of Vit-D, it is wise for medical professionals to exercise caution until randomised clinical trials have produced adequate data to establish the association. For curing of “COVID-19” problems, high dosages of “vitamin D” are not currently advised due to

insufficient evidence. For the time being, doses below the IOM's 4000 IU/day Upper Tolerable Level are the most judicious.

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