SERUM VITAMIN D LEVELS IN COVID PNEUMONIA PATIENTS ADMITTED TO ICU: CORRELATION WITH SEVERITY AND OUTCOME

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ABSTRACT

Background: The global landscape transformed with the emergence of COVID-19 in 2019, a disease profoundly impacting the respiratory system and cascading into multi-organ involvement. Amidst the quest for effective preventive and therapeutic measures, Vitamin D has garnered attention due to its historical role in immunomodulation, particularly in respiratory viral infections.

Objectives:
1. Assess serum Vitamin D levels in patients with severe and critical COVID-19 pneumonia admitted to the ICU.
2. Establish a relationship between low Vitamin D levels and disease severity as well as clinical outcomes, including hospital stay, discharge, and mortality.

Setting: The study was conducted within the COVID-19 and medical ICUs at Liaquat University Hospital (LUH), Hyderabad.

Study Period: Hospital records from August 20, 2020, to January 20, 2021, were retrospectively collected.

Study Design: A retrospective cross-sectional analysis was employed.

Methodology: A cohort of 186 patients (both genders, aged 25-75) admitted to the ICU for critical COVID-19 pneumonia underwent evaluation. Patient demographics, comorbidities, pre-ICU illness duration, laboratory data (including Vitamin D levels), and imaging records were analyzed using SPSS version 22.

Results: Among the 186 patients, 62.4% were male and 37.6% were female. Notably, 83.3% exhibited Vitamin D deficiency, while 15% showed insufficiency. Gender disparities were observed, with males displaying greater susceptibility ($p < 0.05$). Vitamin D deficiency correlated positively with prolonged hospitalization, mechanical ventilation necessity, and mortality. Gender emerged as a confounding factor, as females displayed higher insufficiency rates (22.8% vs. 10%) and males

exhibited greater deficiency rates (85% vs. 75%), with age and comorbidities not exerting a similar impact.

Conclusion: COVID-19 pneumonia patients admitted to the ICU exhibited notably low Vitamin D levels, aligning with increased morbidity and mortality rates. This underscores the significance of addressing Vitamin D status in the management of severe COVID-19 cases.

Keywords: Vitamin D deficiency, 25-hydroxyvitamin D, SARS-CoV-2, COVID-19 pneumonia.

INTRODUCTION:

The pandemic of covid19 has left the world stunned for more than a year now. The infection caused by severe acute corona virus-2 (SARS-Cov-2) is termed novel because the respective virus behaved differently from its peers. It is highly contagious and was seen to affect a subset of people more severely causing a high death toll[1]. It is not only a cause of severe ARDS but also affects other systems causing myocarditis, thrombotic complications(pulmonary embolism, myocardial infarction, stroke), cytokine release syndrome, acute kidney injury, etc; all due to underlying dysregulated inflammatory response[1]. A lot of speculations have been made regarding the prevention and treatment of covid19. Among many treatment strategies including Hydroxychloroquine, azithromycin, remdesivir, tocilizumab, ivermectin ; researchers have found Vitamin D as a potential preventive and treatment option for Covid pneumonia[2]. One of the several roles of vitamin D in our bodies is immunomodulation and secretion of antiviral peptides, leading to an increase in innate immunity and improving mucosal defenses against pathogens.[3,4] It stimulates the expression of cathelicidins and beta defensins in respiratory epithelia to prevent against pathogen invasion.[5,6] Due to its effect on immunomodulation of IL-6 levels, vitamin D has been hypothesized to be useful in place of tocilizumab which is IL-6 receptor blocker, thereby reducing financial burden on hospitals as the latter is an expensive yet potentially effective treatment option.[7] Many studies on impact of low vitamin D on viral infections have been carried out in temperate countries where vitamin D deficiency is prevalent due to cold less sunny weather.[8,10] Very few studies have been done in tropical countries especially Pakistan in this regard, one such study done in Quetta, Baluchistan, a comparatively colder place, showed a significant association of Vitamin D deficiency and severe Covid pneumonia[9].

This study was intended to determine the vitamin D levels in people of Sindh suffering from severe Covid pneumonia, as little data is available on this issue in this part of Pakistan. Here we investigated 186 patients admitted to ICU for covid pneumonia and studied the association between vitamin D levels at admission and correlated it with outcomes, and possible confounding by age, gender and vit D impacting comorbidities.

Since Covid has acted almost similarly in Pakistan as around the world breaking the barriers of demography and temperature, therefore relating it with vitamin D levels can help us understand and manage it in a better way.

MATERIALS AND METHODS

We conducted a retrospective cross-sectional study over four months (August 20 to December 25, 2020) on 186 consecutive patients admitted to the COVID and Medical ICUs at Liaquat University Hospital, Hyderabad. Informed consent was obtained from the ICU Director, and ethical approval was secured from the Ethical Review Committee (ERC). Non-probability consecutive sampling was employed, with males and females aged 25-75 years suffering from severe or critical COVID pneumonia included. Those with dementia, cancer, or pregnancy were excluded. Data were collected from previous hospital records, including demographic details, comorbidities, illness duration, PCR/antibody test results, blood tests (CRP, LDH, Ferritin, ProBNP, D-dimers), serum vitamin D levels, and imaging evidence of pneumonia. Outcomes were classified as prolonged hospital stay, discharge, or death, with stratification addressing confounders. No data on prior vitamin D supplementation were available. The correlation between serum vitamin D levels, severity, and patient outcomes was analyzed.

Data Analysis

Data was analyzed using SPSS version 22. Statistical analyses was carried out using the Pearson Correlation Coefficient Calculator. P value of less than 0.05 was considered as significant. Data is presented in graphs, tables and pi charts format. Confidence interval is taken as 95%. Data (not normally distributed) are expressed as medians (interquartile ranges [IQRs]), and the Mann-Whitney test was used to test statistical differences between groups. Proportions for categorical variables were compared using the chi square (χ^2) test. Multivariate analysis was done by logistic regression or multiple regression as indicated. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with bivariable logistic regression for assessment of demographic characteristics and comorbidities associated with 25(OH)D deficiency and for assessment of demographic characteristics, comorbidities, vitamin D status, imaging findings on admission associated with survival outcome.

RESULTS

A total of 186 patients were included in this study. Out of 186 COVID pneumonia patients admitted to the ICU, 116 (62.4%) were male and 70 (37.6%) were female. COVID-19 antibodies were positive in 165 patients, including 110 males (59.1%) and 55 females (29.6%). The median serum Vitamin D level was 16.6 ng/ml, with 155 (83.3%) patients being deficient, including 102 males (54.8%) and 53 females (28.5%). Comorbidities were prevalent, with 148 patients (79.6%) having diabetes, 93 (50.0%) being obese, and 29 (15.6%) suffering from ischemic heart disease. Regarding BMI, 93 (50.0%) were obese and 81 (43.5%) were overweight. Inflammatory markers were elevated, with a median NLR of 25, CRP of 40 mg/dl, and ferritin of 1000 ng/ml. The mortality rate was 76.3% (142 patients), with 98 males (53%) and 44 females (23.7%) dying, while 20 patients (10.8%) survived with a short ICU stay and 24 (12.9%) survived with prolonged hospitalization. **Table 1**

In this study, the majority of COVID pneumonia patients admitted to the ICU presented with bilateral (B/L) fluffy peripheral infiltrates on chest X-ray, observed in 98% of the cases (n=182). Only a small fraction of the patients, 2% (n=4), exhibited single consolidation. No patients were reported with other types of findings. These results highlight that bilateral peripheral infiltrates are the predominant radiological feature in critically ill COVID-19 patients, which may indicate the severity and diffuse nature of lung involvement in this population. **Table 2**

In this study involving 186 COVID-19 pneumonia patients admitted to the ICU, 83.3% (155) had Vitamin D deficiency, 15.1% (28) had Vitamin D insufficiency, and only 1.6% (3) had normal Vitamin D levels. The median age of patients with Vitamin D deficiency was 60 years (IQR 50-65), while those with insufficiency and normal levels had median ages of 56 years (IQR 50-58) and 52 years (IQR 40-55), respectively (p = 0.26). A significant gender disparity was noted, with males making up 65.8% (102) of the Vitamin D-deficient group compared to 42.9% (12) in the insufficiency group and 66.7% (2) in the normal group (p = 0.009). Among the comorbidities, diabetes mellitus (DM) was prevalent in 90.3% (140) of the Vitamin D-deficient group, followed by obesity in 40.6% (63), ischemic heart disease (IHD) in 15.5% (24), and chronic lung disease in 3.2% (5), though these showed no statistically significant association with Vitamin D levels (p > 0.05). Multivariate analysis revealed that male gender (OR 2.43, 95% CI 1.32-4.50, p = 0.0046) was significantly associated with Vitamin D deficiency, while other factors such as age, DM, obesity, IHD, and chronic lung disease did not show a significant correlation. **Table 2**

**Table 1: Demographic, Lab Data and Outcome of Patients Stratified for Gender (n=186)**

CHARACTERISTICS	FINDINGS		
Total patients	186		
Males	116		
Females	70		
Covid pcr +	All		
Covid antibodies +	110 (males), 55 (females)		
Serum Vit D levels (median IQR)ng/ml	16.6 (10.6-24.6)		
	Total	**Males**	**Fem ales**
Symptomatic days prior to ICU admission (days-median)	8+/-5	12	7
Comorbidity			
DM	148	90	57
Obesity	93	53	40
Chronic lung disease	7	5	2
IHD	29	19	10
s.Vitamin D levels			
Normal	3	2	1
Insufficient	28	12	16
Deficient	155	102	53
Covid19 antibodies positive	144	90	54
BMI (kg/m^2)			
Normal	12	8	4
Overweight	81	55	26
Obese	93	53	40
Socio economic status			
Low income	10	6	4
Middle income	66	41	25
High income	110	69	41
AGE (median) in yrs	60$^{+/-5yrs}$	59.8	55.8
Haemoglobin(Hb) in g/dl - mean	12$^{+/-2}$	13.5	11.8
Inflammatory markers			
NLR (median)	25$^{+/-5}$	27	21
CRP(median) in mg/dl	40$^{+/-5}$	41	38
s.Ferritin(median)in ng/ml	1000$^{+/-500}$	1200	950
Outcome			
Survived (discharged with short ICU stay <3weeks)	20	9	11
Death (with/without need of MV+/- prolonged hospital stay)	142	98	44
Survived with prolonged hospital stay (>30days needing NIV/MV, discharged on home oxygen)	24	9	15

Table 2: CXR findings in percentage (n=100)

CHEST XRAY FINDINGS	% OF PATIENTS (n)
B/L Fluffy peripheral infiltrates	98 (182)
Single consolidation	2 (4)
other	0

Table 3. Clinical Characteristics of Patients with Vitamin D Deficiency, Insufficiency, and Normal Levels (n=186)

Characteristics	Vitamin D deficiency	Vitamin D insufficiency	Normal Vitamin D	*p-value*
All (n=186)	155	28	3	
Age median(IQR) yrs	60 (50-65)	56(50-58)	52(40-55)	0.26
Gender				
Male	102	12	2	0.009
Female	53	16	1	
Comorbidities				
DM	140	6	2	0.99
Obesity	63	13	3	0.56
IHD	24	4	1	0.7
Chronic lung disease	5	2	0	0.9
Multivariate analysis OR(95% CI)				
Age	1.00 (0.97-1.02)			.6601
Male gender	2.43 (1.32-4.50)			.0046
DM	0.99 (0.42-2.34)			.9798
Obesity	0.89 (0.41-2.12)			.8798
IHD	0.87 (0.38-1.99)			.7441
Chronic lung disease	0.69 (0.30-1.61)			.3880
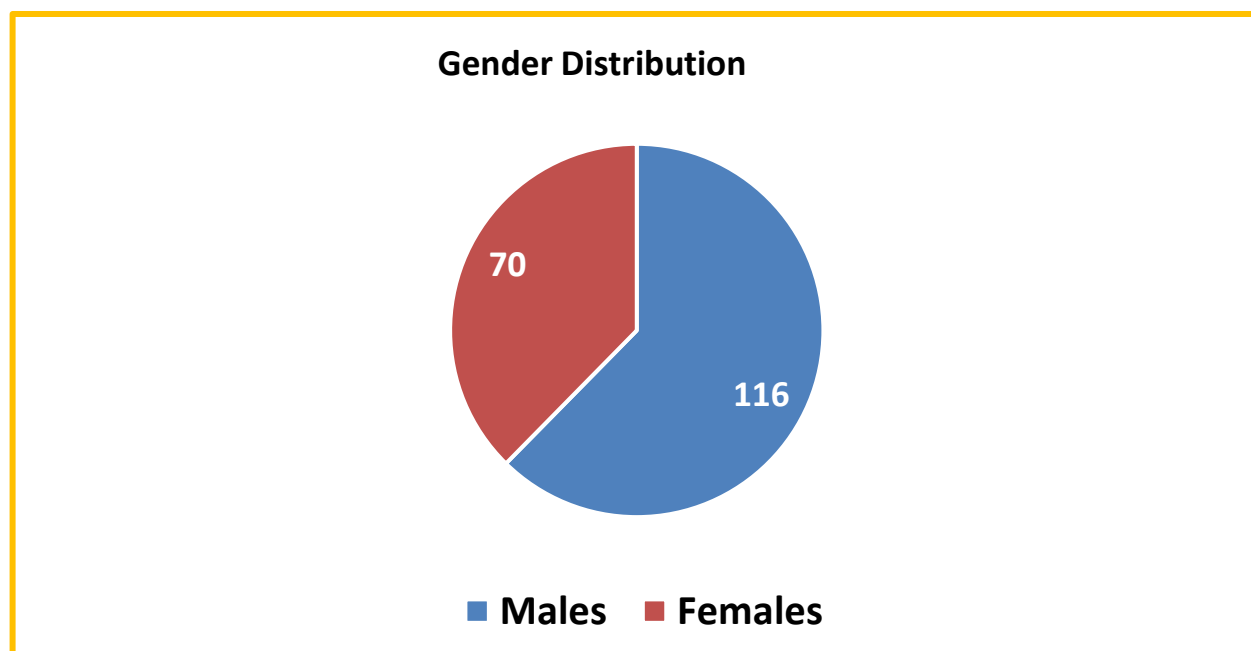
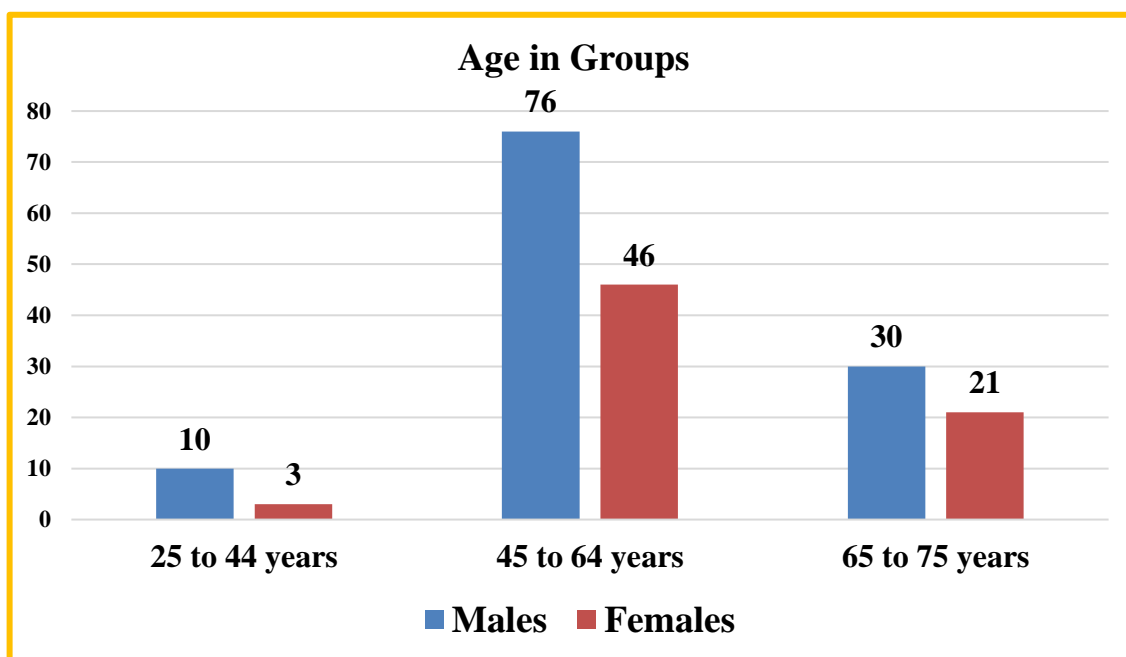**Fig 1: Number of patients in terms of gender distribution (n = 186).**

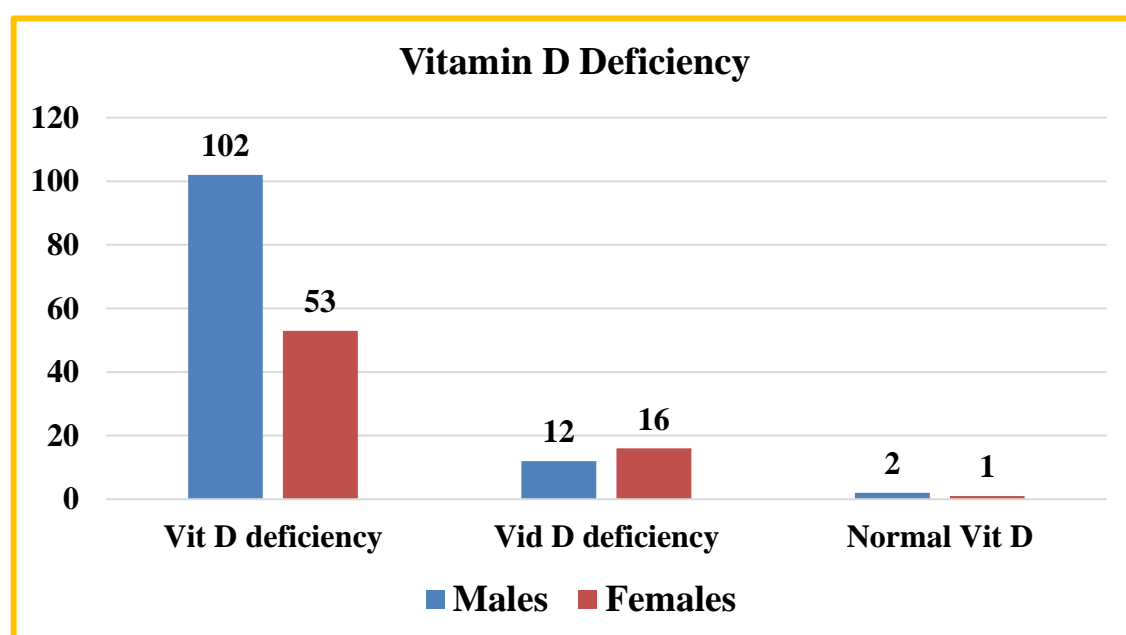**Fig2: Age distribution among males and females. Majority of patients were between 45-64 yrs group.**

Fig 3: Gender distribution among vitamin D deficient, insufficient and normal groups.
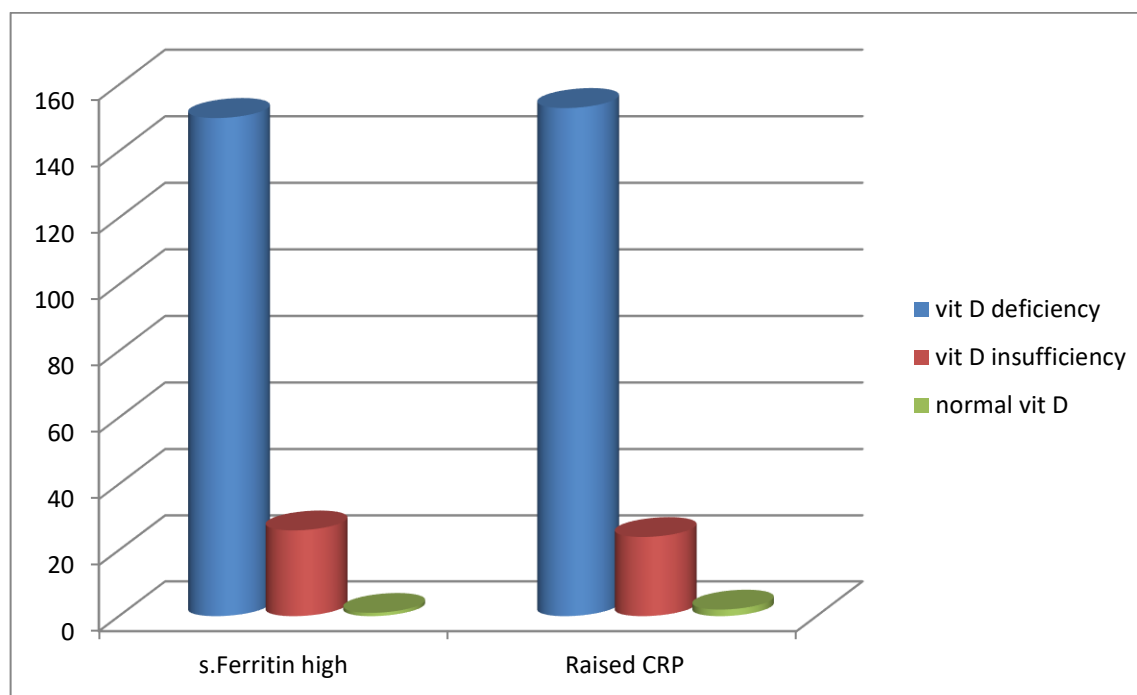
Fig 4: correlation of high serum ferritin and raised CRP with vitamin D status. By High s. ferritin is >1000 ng/ml and raised CRP= >5mg/dl according to lab cut off value.

DISCUSSION

Vitamin D has always been a central discussion point for respiratory viral diseases. Our study aimed to find vitamin D levels in severe covid pneumonia patients admitted to ICU and its correlation with outcomes. Overall vit-D deficiency (s.25[OH]D <20ng/ml) was present in 155 (83.3%) patients, it was positively correlated with increased hospital stay, need of mechanical ventilation and death (odds ratio [OR], 3.87; 95% confidence interval [CI], 1.30-11.55). The finding of high prevalence of vitamin D deficiency in severe covid pneumonia was in concert with study by Orchard L. et al which also demonstrated similar results[12]. Also , Panagiotou also found vitamin D deficiency was more common in patients admitted to the ICU[13]. Our study showed that the deficiency was more prevalent among males and so was a high mortality but at the same time this could be because of high number of male patients in our study group but at the same time male sex hormones, concurrent diseases, behavioral differences, and more exposure of men to pathogens may play a role[14].

The deficiency was also pronounced among those with comorbidities especially Diabetes mellitus. Our study showed that other quartiles of vit. D deficiency were lesser prevalent in ICU patients with severe covid matched the study by Vasheghani M. et al.[13] Our study showed a negative correlation between vit. D levels and inflammatory markers. In the present study, 83.3% percent of patients had 25(OH)D less than 20 ng/ml. However Pakistan is a sunny country, vitamin D defciency is common in all age groups and surprisingly in males as well. The reason is the life style changes with more time spent indoors due to hot weather and more air condition use, lack of intake through food, reduced synthesis of this vitamin in the skin, type of clothing, and no or less supplementation on a national level. We also noticed an incidental correlation between a higher economic status with severity of covid pneumonia irrespective of vitamin D status, which needs study on a larger population sample. Vitamin D supplementation in past medical history was not documented. Whether vitamin D supplementation could improve severity and outcomes in covid pneumonia, remains a speculation because in a few clinical trials, vitamin D administration has reduced the risk of COVID-19 disease, its morbidity, and mortality[16-18]. In a retrospective study, people with a history of a low mean level of 25(OH)D were more likely to develop COVID-19 disease than those with a higher level of 25(OH)D (11 vs. 25 ng/ml, respectively)[19]. Although in another study, a high dose of vitamin D had no impact on the course of COVID-19 disease[20].

A number of studies suggest the link between 25(OH)D levels and COVID-19 disease. In a cohort study of 185 patients with COVID-19 within 66 days, 50% were admitted, 28 required mechanical ventilation, and 16 died. When adjusted for age, gender, and comorbidities, 25(OH)D< 12 ng/ml was associated with a higher risk of death (HR 14.73, 95% CI 4.16–52.19, P<0.001).[21] In a retrospective cohort study, the relative risk and predicted rate of COVID-19 disease was higher in patients with vitamin D defciency than in those with sufficient vit D [1.77 and 1.8 times, respectively)[22]. Vit D defciency may also increase the risk of COVID-19 and disease severity and mortality. In a meta-analysis, a serum level of 25(OH)D< 30 ng/ml was associated with an increased rate of hospital admissions and the mortality rate from COVID-19 (OR 1.82 for both)[23]. The mortality rate 10 days after admission in patients with COVID-19 pneumonia and severe vitamin D deficiency [25(OH)D < 10] ng/ml was 10 times higher than other group (25(OH)D ≥ 10 ng/ml)[24]. Some studies found no relationship between the serum level of 25-OH D and the severity of COVID-19 pneumonia and mortality. In a retrospective population-based study in Brazil, 14,692 people were studied and their serum levels of 25(OH)D and RT-PCR test for COVID-19 was done[25]. There was non-significant difference between PCR positive and PCR negative individuals in mean 25(OH)D quartiles and the prevalence of vitamin D deficiency. A retrospective cohort study on hospitalized 347 covid patients in Italy, showed similar results[26].

The immune system, during severe covid pneumonia does not have the proper response to prevent the multiplying and progressing virus infection. This is because cytokine storm occur due to the release of excessive inflammatory factors[27]. Some of the pathways and mechanisms explaining the relationship between Vitamin D and COVID-19 severity and outcome are mentioned here.
Vitamin D has an antibacterial and antiviral property by regulating innate and adaptive cellular immunity, and physical barriers[28]. Vitamin D produces antimicrobial peptides (AMPs) such as cathelicidins and defensins by activating immune cells. A primary form of Cathelicidins (LL-37) inactivate viruses such as the Influenza A virus by destroying envelope proteins[29]. The "cytokine storm" due to severe COVID-19 releases inflammatory cytokines such as IL-6, IL-8, CRP and ferritin without the control of the immune system. These cytokines damage the integrity of the lungs by causing inflammation, leading to pneumonia, setting up a vicious cycle of inflammation and tissue destruction[30]. IL-6 increases the severity of COVID-19 by rearranging the angiotensin-converting enzyme (ACE2) receptors and inducing macrophage cathepsin L which cleaves the S1 subunit of the coronavirus spike glycoprotein.

This is essential for the coronavirus to enter human host cells, a formation of the endosome membrane of host cell for virus, and release of viral RNA[31]. Vitamin D has immune-modulatory effect, reducing production of pro-inflammatory markers. Vitamin D supplementation has reduced interleukin-6 levels in several clinical trials[32]. Vitamin D may reduce the risk of ARDS COVID-19 related morbidity by raising ACE2 levels. The SARS-COV-2 virus binds to the ACE2 receptor expressed on the surface of lung epithelial cells and causes over-accumulation of angiotensin II by ACE2 down regulation. The active metabolite of vitamin D, calcitriol, increases ACE2 expression in the lungs in animal studies. By this Vitamin D replacement may reduce lung damage by increasing ACE2 expression and synthesis of α-1-antitrypsin by CD4+T cells, which in turn is critical for lung integrity and repair and is required for the further production of anti-inflammatory interleukins such as IL-10. Vitamin D improves endothelial dysfunction by reducing the oxidative stress of free oxygen radicals, TNF-alpha and interleukin-6 and suppressing the NF-κB pathway[33].severe covid causes endothelia dysfunction leading to vascular inflammation and increased coagulation. Vitamin D stimulates the proliferation and migration of alveolar epithelial cells type II and reduce their apoptosis. It also inhibits the mesenchymal transition of an epithelial cell induced by TGF-β[34]. In COVID-19, the function of type II pneumocytes is impaired, decreasing surfactant concentration at the alveolar surface, leading to alveolar collapse. In some studies, 1α, 25 (OH) 2D has increased the production of surfactant and may prevent lung alveolar collapse[35-37]. Certain studies have shown that acute inflammatory disease can

decrease serum levels of vit D but these changes were temporary and short-lived and resolved within 24–48 h. There is a significant diurnal and seasonal fluctuation (20%) in the serum level of 25(OH)D in each person during the day with maximum levels observed in the middle of the day and the summer and autumn. Due to this daily fluctuation, the sampling time makes a significant error in assessing the serum level of 25(OH)D[36-38]. This can change the results.

We would like to acknowledge the limitation of this study. This was a single centric retrospective cross sectional study. The recorded Vitamin D levels were only done once after admission. Sample size is also not adequate so as to declare findings as final. We also didn't take into account the vitamin D binding globulin which is reduced in many severe diseases. The blood samples for vit D levels were taken from August to December, as vitamin D levels are lower during the winter and spring as compared to summer and autumn, the timing of the study may affect the results obtained [37].

We understand that judgments based on these limited studies are not final. Most of such studies are observational, single centric, retrospective and with inadequate sample size with limited human and financial resources and a lack of enough time. Because time factor in project design and implementation, data collection, analysis, and publishing research results impact the quality of researches, therefore we suggest multinational, multicenter, double-blind randomized clinical trials or cohort studies be conducted to clarify the issue.

Nevertheless, our study does provide an insight to the prevalent vitamin D deficiency among the tropical sunny population sample and it's possible association with poor outcome in covid pneumonia. This raises a possibility of therapeutic role of vitamin D, for which further studies are needed. Lastly it will help community and health care professionals to understand the importance of vit D for a healthy life.

CONCLUSION

The vitamin D levels were significantly low in Covid pneumonia patients, admitted to ICU, and were associated with high morbidity and mortality. Overall vit-D deficiency was present in 83.3% of the patients admitted to ICU for covid pneumonia. Vit-D insufficiency was present in 15%. Males were more affected than females with confidence interval of <0.05. Vit D deficiency was positively correlated with increased hospital stay, need of mechanical ventilation and death. Vitamin D deficiency and its correlation with outcome were not confounded by age or comorbidities but gender seemed to have an impact with females being more on insufficient side (22.8% vs 10%) and more males in deficient group (85% vs 75%).

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