ORIGINAL ARTICLE

# A Study on Clinical Correlation of Vitamin D3 (25 Hydroxyvitamin D) Status on Course and Outcome of Hospitalised Cases of Covid-19

**Bal Kishan Gupta[1], Dinesh Kumar Mehra[2], Jigyasa Gupta[3], Vijay Puniya[4], Anjli Gupta[5]**

**ABSTRACT**

*Introduction:* Meta-analysis of various studies has shown association of vitamin-D3 status with prevention, morbidity and mortality associated with respirator-tract infection. Morbidity and mortality associated with Covid-19 infection is mainly because of respiratory-tract involvement. Therefore, this study was planned to evaluate vitamin-D3 status and its clinical significance in hospitalised cases of Covid-19.

*Methods:* This study was conducted in the department of medicine, S.P. Medical College and AGH, Bikaneron hospitalized, RT-PCR confirmed cases of Covid-19 (study group). They were compared with normal healthy subjects living in the same vicinity with history of contact with covid-19 positive patients (control group). All patients were treated as per ICMR guidelines and followed up during hospital stay.

*Results:* Our study shows mean vitamin-D3 was significantly low in study group (16.16±7.15ng/ml) as compared to control group (34.00 ±6.30ng/ml; p<0.0001). Hypovitaminosis D was present in 94.4% of the cases. The level of vitamin D3 was found to be correlated significantly with severity of covid-19 (Mean vitamin-

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D317.03±7.03ng/ml in mild, 18.70±6.61ng/ml in moderate and 11.22±6.05ng/mlin severe cases; p<0.03), comorbidities (12.42±5.74ng/ml vs 16.84±7.37ng/ml; p<0.01), requirement for mechanical ventilation (12.07±5.53ng/mlvs16.53±7.15ng/ml; p<0.003), hospital stay (17.99±6.89ng/ml in ≤7 days, 15.74±7.03ng/ml in 8-15 days, 15.81±5.45ng/ml in >15 days; p<0.006) and poor outcome (11.22±6.05ng/mlvs 16.24± 7.06ng/ml; p<0.002). Vitamin-D3 status was also significantly correlated with CRP-titre (p<0.04), LDH (p<0.001), Trop-I (p<0.02) and Pro-BNP level (p<0.03).

*Conclusion:* Our study shows vitamin-D3 may have preventive role in covid-19 infection. Hypovitaminosis-D is associated with high morbidity and mortality. Further studies are required to evaluate the benefit of vitamin-D3 supplementation in such cases.

**KEYWORDS**

• Covid-19 • Vitamin D • 25 Hydroxy Vitamin D • ARDS • D3, SARS-CoV-2

## INTRODUCTION

Coronavirus Disease-2019 (COVID-19) is caused by infection due to Severe Acute Respiratory Syndrome Corona-Virus-2 (SARS-CoV-2). This virus has high transmissibility, mainly through droplets issued with the phonation and breath or by direct contact. India reported the first confirmed case of the corona virus infection on 30 January 2020. SARS-CoV-2 was new to the human immune system and so there was no underlying existing natural immunity against it. SARS-CoV-2 infects respiratory epithelial cells and clinical spectrum of the disease ranges from completely asymptomatic form to respiratory symptoms like fever, cough, chest pain, sore throat and in severe cases may lead to severe acute respiratory distress syndrome (ARDS) requiring ventilator support and multi-organ failure.[1]

The immune system becomes vital once an individual is exposed to an infectious agent. Vitamin plays a role in infectious disease management by both direct and indirect mechanisms.[2] Vitamin D perceives a crucial role in regulating mitochondrial activity including the redox homeostasis and protection against oxidative stress.[3] Consequently, Vitamin D acts as the key holder in terms of modulating systemic inflammation, oxidative stress, and mitochondrial respiratory functions. Vitamin D is responsible for protection against excess, ROS production or cellular damage.[4]

Worldwide, Vitamin D deficiency is becoming a public health concern and its deficiency is associated with several adverse health outcomes.[5,6] As an antiviral agent, Vitamin D may constitute an inexpensive prophylactic option either by itself or as a synergistic agent during the treatment of different viral infections.[7]

Vitamin D supplementation to raise serum 25(OH)D concentrations can help in decreasing the rate of many different infections, including viral.[8-11] Most countries have existing health recommendations as to Vitamin D intakes, yet a significant proportion of populations are often deficient and/or insufficient. Meta-analysis of various studies has shown that vitamin D effectively decreases risk of respiratory infections and decreased vitamin D3 level found to be associated with high risk and severity of respiratory infections.[2-5]

## METHODS

This prospective cross sectional study was conducted in the department of medicine, S.P. Medical College and AGH, Bikaner on hospitalized, RT-PCR confirmed cases of Covid-19 (study group), they were compared with normal healthy subject living in same vicinity with history of contact with Covid 19 positive patients (Control group). Study group was further classified according to severity of the infection as Asymptomatic, Mild, Moderate and severe Covid. Ethics committee approval was taken before start of the study, all participating subjects were explained about the study and informed consent was taken. All patients were subjected to detailed clinical examination and relevant investigation as per proforma including complete blood count, renal function test, liver function test, fasting

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blood sugar, CRP titre, LDH, pro-BNP, Serum Troponin I and vitamin D3 assay. Vitamin D3 assay was done in all the cases by automated chemiluminescence immunoassay (CLIA) using uniceldxI 800 machine (Beckman Coulter). Vitamin D3 Levels were classified into three categories as per US Endocrine Society (2011)[12] criteria: Deficient: - Vitamin D3 level < 20 ng/ml, Insufficient: - Vitamin D3 level 20-30 ng/ml and Sufficient: - Vitamin D3 level >30 ng/ml.In our study low level of 25(OH)D (Hypovitaminosis D) means levels ≤30 ng/ml.

## STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Chi-square test was done for qualitative variables and Student t-test was used for quantitative variables. Pearson's Correlation test was done to evaluate correlation between outcome and various parameters and Multiple Linear Regression Analysis was done to predict effect of vitamin D3 in relation to various variables. p<0.05 was considered as statistically significant.

## RESULTS

Present study included a total of 126 cases of covid (mean age 43.45 ±17.10 years), out of which 83 (65.87%) were males (mean age 43.26±17.03 years, ranging 19-76 years) and 43 (44.13%) females (mean age 42.69±16.30 years, ranging 21 to 79 years) and they were compared with 40 normal healthy subjects (mean age 44.25 ±14 .86 years), 24 males (60%, mean age 42.76±11.0 ranging 21-68 years and 16 females (40%, mean age 41.44±13.50 years ranging 22-73 years) of control group who were the family members of the study group living in the same vicinity and exposed to same environment and risk of getting covid infection.

Mean vitamin D3 in study group was 16.16±7.15 ng/ml (ranging4.55-32.9ng/ml) while in control group it was 34±6.30 ng/ml (ranging 24-46 ng/ml) (p<0.0001). 94.4% cases Iin the study group were having low status of vitamin D3 as compare to 32.5% in control group. All females in the study group had low status of vitamin D3. Mean age of patients with low vitamin D3 (<30ng/ml)

was 42.52±16.68 years, whereas mean age of patients with sufficient vitamin D3 (>30ng/ml) was 59.14±17.62 years. (p<0.01).

Analysis of vitamin D3 status in relation to severity of covid: Out of total 126 cases 48 cases were asymptomatic, 79.17% of them were deficient, 14.58% insufficient and 6.25%, were having sufficient vitamin D3 status (mean 15.60±7.21ng/ml). 55 cases were mildly symptomatic out of them 70.91%, 23.64% and 5.45% cases were having deficient, insufficient and sufficient vitamin D3 status respectively (mean 17.03±7.11ng/ml). 8 cases were in moderate category out of them 75%, 12.5% and 12.5% cases were having deficient, insufficient and sufficient vitamin D3 status respectively (mean 18.70±6.61ng/ml) while 15 cases were in severe category, all of them had low status of vitamin D3 (86.67%Deficient and 13.33% were having insufficient vitamin D3 status; mean 11.22±6.05ng/ml) (p<0.001).

Vitamin D3 status in relation to different presenting symptom is shown in table 1. Fever was the most common presenting symptom out of which 93.02% had low status of vitamin D3. Patients presenting with sore throat, nausea, headache, sneezing nasal discharge and loss of smell and taste all of them had low vitamin D3 status (100%) while 97.44% cases with cough and 94.14% cases with breathlessness had low status of vitamin D3.

**Table 1:** Vitamin D3 status in relation to presenting symptoms

| Symptoms | No.of Cases (%) (n=126) | No of cases (%) with Low Vitamin D3 (≤30ng/ml) |
|---|---|---|
| Asymptomatic | 48 (38.10%) | 45 (93.75%) |
| Fever | 43 (34.13%) | 40 (93.02%) |
| Sore Throat | 13(10.32%) | 13 (100%) |
| Nausea | 8 (6.35%) | 8 (100%) |
| Cough | 39 (30.95) | 38 (97.44%) |
| Headache | 13(10.32%) | 13 (100%) |
| Nasal Discharge | 7(5.56%) | 7 (100%) |
| Breathlessness | 17 (13.49%) | 16 (94.12%) |
| Anosmia | 3(2.38%) | 3 (100%) |
| Sneezing | 12(9.52%), | 12 (100%) |

Our study shows low status of vitamin D3 in 92.11% of the cases of covid with comorbidity and in 95.45% of the cases without any comorbidity. Distribution of cases according to vitamin D3 and comorbidities is shown in table 2. We also found significantly low levels of vitamin D3 in covid cases with various organ dysfunction as shown in table 3.

**Table 2:** Distribution of cases according to vitamin D3 status and comorbidities

| Comorbidity | No of cases (%) (total n=126) | Vitamin D3 status | | | | | | | |
| | | <20 (n=96) | | 20-30 (n=23) | | >30 (n=7) | | ≤30 (n= 119) | |
| | | No | % | No | % | No | % | No | % |
|---|---|---|---|---|---|---|---|---|---|
| DM | 18(14.29) | 15 | 83.33 | 2 | 11.11 | 1 | 5.56 | 17 | 94.44 |
| HTN | 18(14.29) | 12 | 66.67 | 4 | 22.22 | 2 | 11.11 | 16 | 88.89 |
| CAD | 5 (4) | 1 | 20.00 | 2 | 40.00 | 2 | 40.00 | 3 | 60.00 |
| TB | 2(1.6) | 2 | 100.00 | 0 | 0.00 | 0 | 0.00 | 2 | 100.00 |
| Hypothyroidism | 2(1.6) | 2 | 100.00 | 0 | 0.00 | 0 | 0.00 | 2 | 100.00 |
| CKD | 2(1.6) | 2 | 100.00 | 0 | 0.00 | 0 | 0.00 | 2 | 100.00 |
| Seizure disorder | 1(0.8) | 1 | 100.00 | 0 | 0.00 | 0 | 0.00 | 1 | 100.00 |
| Rheumatoid Arthritis | 1(0.8) | 1 | 100.00 | 0 | 0.00 | 0 | 0.00 | 1 | 100.00 |
| Malignancy | 2(1.6) | 2 | 100.00 | 0 | 0.00 | 0 | 0.00 | 2 | 100.00 |
| Comorbidity | 38(30.16) | 29 | 76.32 | 6 | 15.79 | 3 | 7.89 | 35 | 92.11 |
| No Comorbidity | 88(69.84) | 67 | 76.14 | 17 | 19.32 | 4 | 4.55 | 84 | 95.45 |

**Table 3:** Vitamin D3 status in relation to different organ dysfunction

| Organ Dysfunction | Organ Dysfunction | No of cases (%) | mean±SD | p |
|---|---|---|---|---|
| Hepatic | Yes | 38 (30.16)) | 14.09±5.61 | <0.05 |
| | No | 88 (69.84) | 16.94±7.50 | |
| Renal | Yes | 23 (18.25) | 14,11±7.25 | <0.03 |
| | No | 103 (81.75) | 16.62±7.14 | |
| CRP | >6 | 62 (49.21) | 14.68±6.984 | <0.04 |
| | ≤6 | 64 (50.79) | 16.26±7.34 | |
| LDH | >480 | 106 (84.13) | 15.04±7.10 | <0.01 |
| | ≤480 | 20 (15.87) | 18.64±7.59 | |
| Pro-BNP | >300 | 31 (24.60) | 16.01± 7.06 | <0.03 |
| | ≤300 | 95 (75.40) | 17.07±8.37 | |
| Trop I | >19 | 51 (40.48) | 15.08±6.57 | <0.02 |
| | ≤19 | 75 (59.52) | 16.42±7.59 | |

In our study out of total 126 cases, 111 cases did not require ventilation support out of which 93.69% were having low vitamin D3 status, while all the cases (100%) who required ventilation support, invasive or non-invasive ventilation, had low vitamin D3 status (p<0.003). Table 4.

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**Table 4:** Vitamin D3 status and requirement for ventilation support

| Ventilation | No of cases | Vitamin D3 Status | | | | | | | | mean±SD | p |
| | | <20 | | 20-30 | | >30 | | ≤30 | | | |
| | | No | % | No | % | No | % | No | % | | |
| Invasive | 5 (4) | 4 | 80.00 | 1 | 20.00 | 0 | 0.00 | 5 | 100 | 12.07±5.53 | |
| Non- Invasive | 10 (7.9) | 9 | 90.00 | 1 | 10.00 | 0 | 0.00 | 10 | 100 | | <0.003 |
| No Ventilation | 111(88.1) | 83 | 74.77 | 21 | 18.92 | 7 | 6.31 | 104 | 93.69 | 16.53±7.13 | |

Vitamin D3 status and hospital stay: Out of total 110 non-ventilated patients who survived, 37 patients had hospital stay of ≤7 days and out of them 70.27%, 21.62% and 8.11% cases had vitamin D3 levels<20, 20-30 and >30 ng/ml respectively, 62 cases had hospital stay between 8-15 days and among them 79.03%, 14.52% and 6.45% cases had their vitamin D3 <20, 20-30 and >30 ng/ml respectively while 11 cases had hospital stay of >15 days and out of them 63.64%, 36.36% had vitamin D3 <20, 20-30 ng/ml respectively. Mean vitamin D3 level in ≤7 days hospital stay was 17.99±6.89 ng/ml, in 8-15 days it was 15.74±7.03 ng/ml and in >15 days hospital stay it was 15.81±5.45ng/ml (p=0.006). Fig 1.
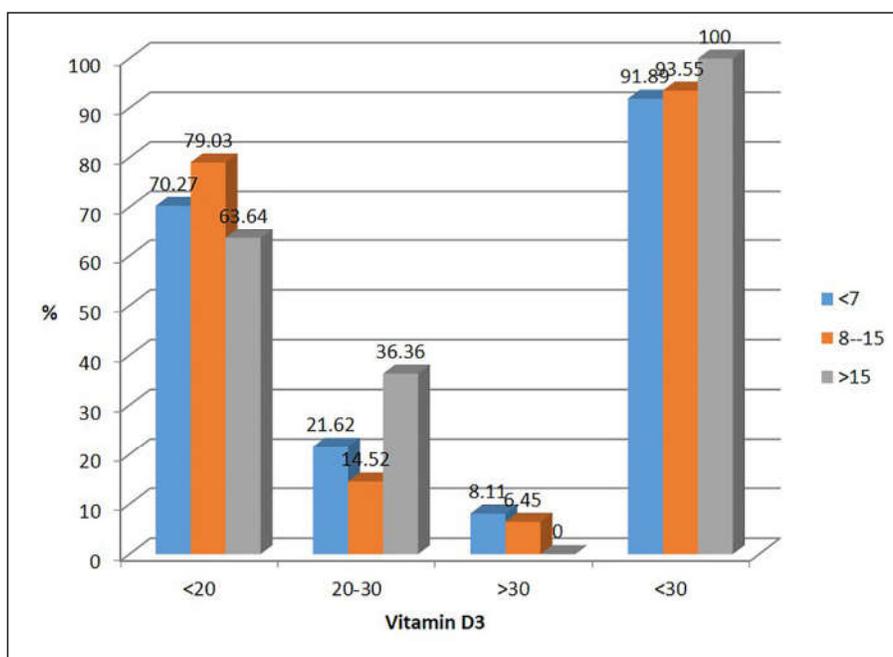


Out of total 126 cases, 6 (4.76%) died, and all of them had vitamin D3 level <20 ng/ml. Mean vitamin D3 level in survived patients was 16.24±7.06 ng/ml as compared to 11.22±6.05ng/ml in died patients (p=0.002). Table 5. Clinical profile of died patients are shown in table 6.

**Table 5:** Vitamin D3 level in relation to outcome

| Outcome | No of Cases | Vitamin D3 status | | | | | | | | mean±SD |
| | | <20 | | 20-30 | | >30 | | <30 | | |
| | | No | % | No | % | No | % | No | % | |
| Death | 6 | 5 | 83.33 | 1 | 16.67 | 0 | 0.00 | 6 | 100 | 11.22±6.05 |
| Survived | 120 | 91 | 75.83 | 22 | 18.33 | 7 | 5.83 | 119 | 94.44 | 16.24±7.06 |
| Total | 126 | 96 | | 23 | | 7 | | 119 | | p=0.002 |

**Table 6:** Clinical profile of cases died in Covid-19

| | Died Patient | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| Patient ID | C-42809 | C-36115 | C-33174 | C-39388 | C-44781 | C-52875 |
| Age | 60 | 66 | 60 | 46 | 60 | 63 |
| Gender | Male | Female | Male | Male | Female | Male |
| Duration of Illness | 8 | 8 | 10 | 2 | 10 | 7 |
| Category | Moderate | Severe | Severe | Severe | Severe | Severe |
| Comorbidity | Nil | HTN | DMT2 | CKD MHD | CKD | DM/HTN |
| X-ray Finding | B/L Pneumonitis | B/L Pneumonitis | B/L Pneumonitis | B/L Pneumonitis | B/L Pneumonitis | B/L Pneumonitis |
| Vitamin D3 | 19.29 | 12.11 | 16.89 | 4.65 | 4.99 | 9.4 |
| Mechanical Ventilation | No-ventilation | Invasive | Invasive | Invasive | Invasive | Invasive |

We also evaluated correlation of vitamin D3 level with Cycle Threshold (CT) value in RT-PCR of the cases and found that CT value of 28 and 29 had highly significant correlation with vitamin D3 status ($p < 0.005$) while CT value of 25, 27 and 30 had statistically significant correlation with vitamin D3 ($p < 0.05$). Table 7.

**Table 7:** Vitamin D3 status in relation with CT Value

| CT Value | Total no. of patients | Vitamin D3 | | | | Mean Vit. D3 | P |
|---|---|---|---|---|---|---|---|
| | | **<20** | **20–30** | **>30** | **<30** | | |
| 20 | 1 | 0 | 1 | 0 | 1 | 22.38 | 0.83 |
| 21 | 1 | 1 | 0 | 0 | 1 | 18.61 | 0.29 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | – |
| 23 | 2 | 2 | 0 | 0 | 0 | 10.10 | 0.43 |
| 24 | 6 | 5 | 1 | 0 | 6 | 15.95 | 0.38 |
| 25 | 9 | 8 | 1 | 0 | 9 | 12.70 | 0.02 |
| 26 | 12 | 6 | 6 | 0 | 12 | 18.18 | 0.01 |
| 27 | 16 | 14 | 2 | 0 | 16 | 14.69 | 0.04 |
| 28 | 13 | 8 | 2 | 3 | 10 | 19.90 | 0.001 |
| 29 | 25 | 21 | 3 | 1 | 24 | 16.71 | 0.001 |
| 30 | 7 | 5 | 2 | 0 | 7 | 15.19 | 0.04 |
| 31 | 3 | 3 | 0 | 0 | 3 | 14.86 | 0.06 |
| 32 | 2 | 2 | 0 | 0 | 2 | 10.31 | 0.08 |
| 33 | 1 | 0 | 1 | 0 | 1 | 23.35 | 0.12 |
| 34 | 1 | 1 | 0 | 0 | 1 | 8.77 | 0.18 |

On Pearson's correlation analysis, LDH ($p < 0.001$) and Vitamin D3 ($p < 0.002$) were found to have highly significant correlation with poor outcome while comorbidity, blood urea, serum Creatinine, ventilation support, CRP titre, pro-BNP, Trop-Ihad statistically significant correlation ($p < 0.05$). Parameters like Age, sex, hospital stay, duration of illness did not show statistically significant correlation with outcome. Table 8.

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**Table 8:** Pearson's Correlation between different parameters and outcome

| Parameters | r value | p value |
|---|---|---|
| Category | 0.26 | 0.22 |
| Age | 0.89 | 0.39 |
| Hospital Stay | 0.21 | 0.18 |
| Blood Urea | 0.08 | 0.01 |
| Serum creatinine | 0.06 | 0.05 |
| Vitamin D3 | 0.13 | 0.002 |
| Duration of Illness | 0.01 | 0.27 |
| Ventilation | 0.001 | 0.05 |
| Sex | 0.28 | 0.18 |
| Comorbidity | 0.19 | 0.01 |

| Parameters | r value | p value |
|---|---|---|
| LDH | 0.35 | 0.001 |
| Trop I | 0.23 | 0.02 |
| CRP | 0.18 | 0.04 |
| Pro BNP | 0.31 | 0.03 |
| CT value | 0.69 | 0.18 |

On multiple linear regression analysis of Vitamin D3 Status in relation to various prognostic factor and outcome, we found that Hospital stays, requirement for ventilation, serum creatinine, CRP titre, pro-BNP, Age, Sex, duration of illness and outcome were having highly significant correlation ($p<0.001$) while category of severity, LDH, Trop-I were statistically significant corelated ($p<0.05$). Table 9. Fig. 2.

**Table 9:** Multiple Linear Regression Analysis (ANOVA) in relation to vitamin D3

| Model | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Category | Regression | 607.006 | 1 | 607.006 | 4.510 | .037a |
| | Residual | 10362.606 | 77 | 134.579 | | |
| | Total | 10969.612 | 78 | | | |
| Hospital Stay | Regression | 2871.762 | 2 | 1435.881 | 13.476 | .000b |
| | Residual | 8097.850 | 76 | 106.551 | | |
| | Total | 10969.612 | 78 | | | |
| Ventilation | Regression | 3079.356 | 3 | 1026.452 | 9.757 | .000c |
| | Residual | 7890.256 | 75 | 105.203 | | |
| | Total | 10969.612 | 78 | | | |
| Serum Creainine | Regression | 3129.630 | 4 | 782.407 | 7.385 | .000d |
| | Residual | 7839.982 | 74 | 105.946 | | |
| | Total | 10969.612 | 78 | | | |
| CRP | Regression | 3177.444 | 5 | 635.489 | 5.954 | .000e |
| | Residual | 7792.168 | 73 | 106.742 | | |
| | Total | 10969.612 | 78 | | | |
| LDH | Regression | 1246.38 | 5 | 12468.38 | 9.568 | .04e |
| | Residual | 9723.23 | 73 | 95.46 | | |
| | Total | 10969.612 | 78 | | | |
| Trop I | Regression | 45.59 | 5 | 45.59 | 2.88 | .03e |
| | Residual | 10924.02 | 73 | 85.52 | | |
| | Total | 10969.612 | 78 | | | |

| Model | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Pro BNP | Regression | 784.89 | 5 | 784.89 | 2.98 | .000e |
| | Residual | 10184.722 | 73 | 58.56 | | |
| | Total | 10969.612 | 78 | | | |
| CT | Regression | 28.59 | 5 | 28.59 | 1.29 | 0.18 |
| | Residual | 10941.02 | 73 | 15.44 | | |
| | Total | 10969.612 | 78 | | | |
| Death | Regression | 1289.89 | 5 | 1289.89 | 5.55 | 0.04 |
| | Residual | 9679.722 | 73 | 106.742 | | |
| | Total | 10969.612 | 78 | | | |
| Age | Regression | 3448.735 | 6 | 574.789 | 5.503 | .000f |
| | Residual | 7520.877 | 72 | 104.457 | | |
| | Total | 10969.612 | 78 | | | |
| Sex | Regression | 3653.106 | 7 | 521.872 | 5.064 | .000g |
| | Residual | 7316.506 | 71 | 103.049 | | |
| | Total | 10969.612 | 78 | | | |
| Duration of Illness | Regression | 3824.446 | 8 | 478.056 | 4.683 | .000h |
| | Residual | 7145.166 | 70 | 102.074 | | |
| | Total | 10969.612 | 78 | | | |
| Outcome | Regression | 4730.160 | 9 | 525.573 | 5.812 | .000i |
| | Residual | 6239.452 | 69 | 90.427 | | |
| | Total | 10969.612 | 78 | | | |

a. Predictors: (Constant), category

b. Predictors: (Constant), category, hospital stay

c. Predictors: (Constant), category, hospital stay, ventilation

d. Predictors: (Constant), category, hospital stay, ventilation, screatinine

e. Predictors: (Constant), category, hospital stay, ventilation, screatinine, Serum CRP

f. Predictors: (Constant), category, hospital stay, ventilation, screatinine, Serum CRP, age

g. Predictors: (Constant), category, hospital stay, ventilation, screatinine, Serum CRP, age, sex 1

h. Predictors: (Constant), category, hospital stay, ventilation, screatinine, Serum CRP, age, sex 1, Duration

i. Predictors: (Constant), category, hospital stay, ventilation, screatinine, Serum CRP, age, sex 1, Duration, Outcome
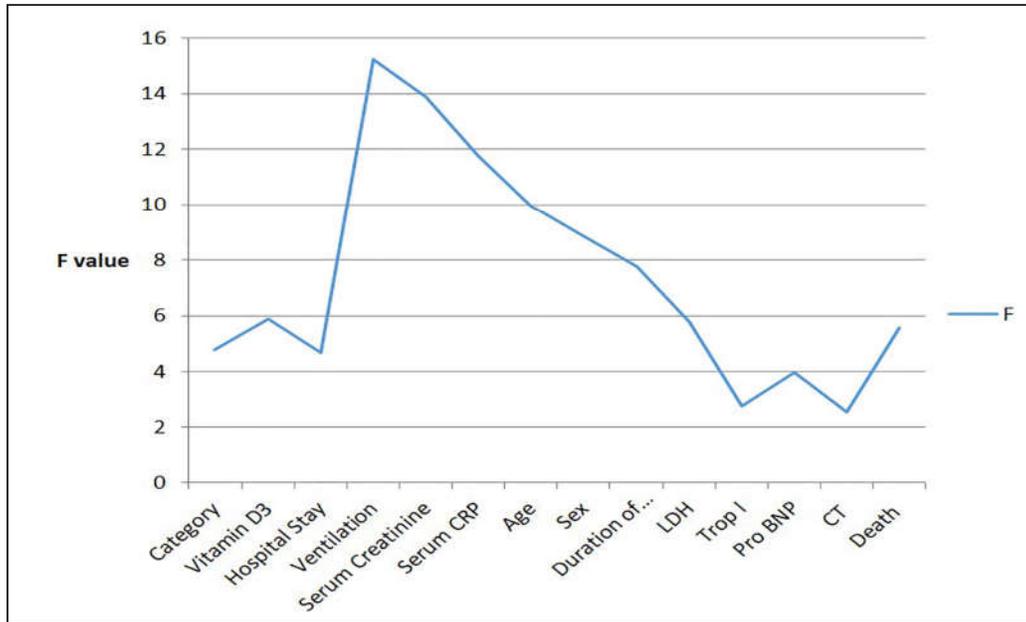
j. Dependent Variable: vitaminD3

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**Figure 2:** Multiple Linear Regression Analysis (ANOVA) in relation to vitamin D3

## DISCUSSION

We found high prevalence (94.4%) of low status of vitamin D 3 in cases of Covid 19. Dror *et al* also reported low status of vitamin D3 in covid cases.[13] We also evaluated vitamin D3 status in healthy subjects living in the same vicinity with the covid-19 cases and found that Vitamin D3 level was significantly low in covid cases as compared to normal healthy subjects although having equal exposure to infection indicating protective role of Vitamin D3 in the development of Covid-19 disease. COVID-19 importantly affects the respiratory tract, and the data supporting a significant effect of vitamin D in preventing and mitigating respiratory tract infections have emerged. A meta-analysis of individual participant data published in 2017 analysed over 11,300 patients from 25 randomized studies and demonstrated the protective effect of vitamin supplementation D on respiratory tract infections.[3] Griffin *et al*[14] demonstrated in their study on data from 348598 UK Biobank participants to correlate historical vitamin D levels checked between 2006 and 2010 with risk for Covid 19 positivity, their analysis showed a significantly lower median vitamin D in those tested positive (43.8nm/l, IQR 28.7-62.6) than in those tested negative for Covid 19 (47.2 nm/l IQR 32.7-62.7; P<0.01). Hernandez *et al*[15] demonstrated significantly low levels of vitamin D in Covid 19 patients as compared

to controls(13.8±7.2ng/ml vs 20.9±7.4 ng/ml, p<0.0001). He found Vitamin D deficiency in 82.2% of Covid 19 cases and 47.2 % of population-based controls (p<0.0001).

We found vitamin D deficiency was more commonly associated with younger age similar to observation made by Jude *et al*[16] demonstrated that Covid 19 patients who were vitamin D deficient were more likely to be younger (age 45.9 years vs 51.0 years; p=0.04).

Our study shows lower levels of vitamin D is associated with higher prevalence of symptoms in covid. Asghar *et al*[17] also demonstrated higher prevalence of symptoms among Covid 19 patients with 25(OH)D <10 ng/mL in comparison to those with 25(OH)D ≥10 ng/mL, including cough with sputum (38.9% [*N*=7/18] versus 13.7% [*N*=10/73]; *P*=0.022), dyspnea/shortness of breath (72.2% [13/18] versus 35.6% [26/73]; *P*=0.005), fatigue (55.6% [10/18] versus 26.0% [19/73]; *P*=0.016), nasal congestion/rhinorrhea (38.9% [7/18] versus 13.7% [10/73]; *P*= 0.022), diarrhea (38.9% [7/18] versus 15.1% [11/73]; *P*=0.043), anosmia (22.2% [4/18] versus 5.5% [4/73]; *P*=0.046), and malaise (50% [9/18] versus 17.8% [13/73]; p=0.007). It has been documented that vitamin D plays a vital role in the regulation of the renin angiotensin system. SARS-CoV-2 is known to exploit the ACE-II receptors to facilitate its entry into the host cell. Vitamin D modulates multiple immune mechanisms to contain the

virus including dampening of viral entry and replication of SARS-CoV-2, suppressing the hyper inflammatory state by increasing anti-inflammatory cytokine levels.

In our study we found that lower levels of Vitamin D3 is associated with increased severity of Covid 19. All the cases of severe category were having low Vitamin D3 status and their vitamin D3 levels were significantly low as compared patients belonging to asymptomatic, mild or moderate category (p<0.001). This indicate that low level of vitamin D3 is associated with increased risk of severity. Hastie *et al*[18] have followed this up with an updated report based on 341484 UK biobank participants again with vitamin D levels checked between 2006 and 2010 and found low vitamin D concentration was associated with severe Covid 19 infection and mortality. In a meta-analysis study by Ben-Eltriki *et al*, they found a potential increased risk of developing severe COVID-19 infection among patients with low vitamin D levels.[19] Jain *et al*[20] also found significantly low levels of vitamin D in severe covid (14.35 ±5.79 vs 27.89±6.21 ng/ml). Maghbooli *et al*[21] demonstrated that there was a significant association between vitamin D sufficiency and reduction in clinical severity in Covid 19 patients, only 9.7% of patients older than 40 years who were vitamin D sufficient succumbed to the infection compared to 20% who had a circulating level of vitamin D level<30 ng/ml. The significant reduction in serum CRP, an inflammatory marker, along with increased lymphocytes percentage suggest that vitamin D sufficiency also may help modulate the immune response possibly by reducing risk for cytokine storm in response to this viral infection.

Dror *et al*[13] showed that patients with vitamin D deficiency (<20 ng/ml) were 14 times more likely to have severe or critical disease than patients with vitamin D >40 ng/ml (OR 14; 95% CI, 4 to 51; p=0.001). Experimental laboratory evidence evaluating the impact of Vitamin D on immunological responses has shown inhibitory effects on the production of pro inflammatory cytokines, including TNF alpha and IL-6, by various mechanisms, including down regulating viral induced NFkB activation.

Our study shows that patient with Vitamin D3 deficiency were more likely to develop respiratory failure and require mechanical support as compared to patient having normal Vitamin D3 status. Shah *et al*[22] showed strong evidence that Vitamin D supplementation in Covid 19 reduces the need for intensive care (Odds ratio: 0.35; 95% CI: 0.28-0.44; p<0.001) and mechanical ventilation (Odds ratio: 0.54; 95% CI 0.411-0.708; p<0.001).Bassatne *et al*[23] showed a trend for an increase invasive ventilation requirement in Covid 19 patients with vitamin D level <20 ng/ml(RR=1.3,95%CI[0.6-2.8]; I²=0%)

When we correlated vitamin D3 status with course of illness during hospitalization in patients who survived and were not put on ventilator we observed that patient with low vitamin D3 status required longer duration for recovery. Gonen *et al*[24] showed that the covid cases who stayed in hospital longer than 8 days were significantly less who had vitamin D treatment compared with the ones that had no vitamin D treatment (p=0.02). Without vitamin D treatment and vitamin D < 30 ng /ml had the 1.9-fold increased risk of hospitalization for longer than 8 days (P=0.007, OR: 1.91, 95% CI: 1.19-3.06). Vitamin D replacement is hypothesized to reduce infection related mortality in intensive care units via increasing hemoglobin concentrations, reducing serum hepcidin concentrations, improving oxygenation on cellular level, and reversing lung damage. Nasiri *et al*[25] showed a significant difference in hospital stay in Covid 19 patients with sufficient and insufficient vitamin D levels (P=0.007). Adjusting vitamin D levels for confounding variables, linear regression underscored significant differences in the association between length of hospitalization and lower vitamin D levels, with a longer stay noted in insufficient groups (P=0.002). vitamin D up regulates ACE 2 expression, which in the lungs has shown protective effect against acute lung injury.

Mortality rate in our study was 4.76% and all of them were having low level of vitamin D3. So, vitamin D3 deficiency was found to be associated with high mortality. Campi *et al*[26] concluded that low vitamin D3 levels in Covid 19 patient was independent predictors of severity and mortality. Gupta *et al*[27] showed vitamin D3 may have preventive role for swine flu infection. Low level of vitamin D3 is associated with high morbidity in terms of increase requirement for mechanical ventilation, multiorgan dysfunction and

long duration of hospital stay. Patients with vitamin D3 deficiency were associated with high mortality in swine flu. But Davoudi *et al* concluded that sufficient vitamin D levels were not found to be protective against adverse clinical outcomes in patients infected with COVID-19.[28] However, Benskin *et al*[29]showed that staying indoor during lockdown could cause increase in Covid 19 fatalities by increasing vitamin D3 deficiency rates. Their study presented primarily biological plausibility evidence overwhelmingly support the assertions that vitamin D sufficiency increases resistance to viral infections and helps prevent every symptom of severe covid 19 that results in fatalities. Their study also demonstrated that vitamin D3 deficiency explains the geographical differences in Covid 19 case and fatality rates. Vitamin D deficiency increase the activity of the X chromosome linked Renin Angiotensin system making vitamin D deficient individuals more susceptible to Covid 19's deadly cytokine storm. Saeed *et al*[30] concluded that lower level of vitamin D3 in admission was strongly evident in patients with severely symptomatic and in mortality of Covid 19 patients when compared with patients who survived. Borsche *et al*[31] showed a negative Pearson correlation of Vitamin D3 level in Covid 19 patients and mortality risk $(r(17)=-.4154, p=0770/r(13)=-.4886, p=0.646)$. For the combined data median (IQR) D3 levels were 23.2 ng/ml (17.4-26.8), and a significant Pearson correlation was observed $(r(32)=-.3989, p=.0194)$. Regression suggested a theoretical point of zero mortality at approximately 50 ng/ml D3. Vitamin D play a fundamental role in regulating both innate and adaptive immune systems, intra cellular vitamin D3 receptor are present in nearly all cell types involved th the human immune response, such as monocytes /macrophages, T cells B cells, NK cells and dendritic cells.

## CONCLUSION

Our study suggests that vitamin D3 may have preventive role for Covid 19 infection. Low level of vitamin D3 is associated with high morbidity in terms of increase requirements for mechanical ventilation, multiorgan dysfunction and long duration of hospital stay. Patients with Vitamin D3 deficiency are also associated with high mortality in Covid 19. Therefore,evaluation for status of vitamin D3 is very important to predict morbidity and mortality. Further studies are required to evaluate the benefit of vitamin D3 in such cases.

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