Original Research Article

A cross-sectional study to explore the correlation of serum Vitamin D3 with laboratory parameters among COVID-19 patients of a tertiary care centre of Uttar Pradesh, India

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Abstract

Background: There have been a few published studies regarding the significance of Vitamin D levels as a predictor of disease severity, amongst other biomarkers like D-Dimer, CRP, and Ferritin in previous disease outbreaks like H1N1 and SARS. Therefore, the present study aimed to explore the association of Vitamin D3 levels with other laboratory parameters including D3 with biochemical, inflammatory markers and CT Value of COVID-19 patients admitted to Government Institute of Medical Sciences, Greater Noida, a tertiary care hospital during pandemic. **Methods:** This was a cross sectional study that enrolled 67 RTPCR positive patients over a period of 3 months (May 2020 to August 2020) after obtaining institutional ethical approval. Blood tests for collected blood samples were performed in an appropriate autoanalyzer after complying internal quality control. The correlation between Vitamin D levels and other lab parameters was assessed using Pearson correlation test. The comparison of quantitative variables (biochemical, inflammatory markers and CT Value) between groups was performed using the student's t test. All statistical analyses were carried out at 5% level of significance and p-value 0.05 was considered significant. **Results :** The present study enrolled 67 COVID-19 patients out of which only 61 patients had validated measurement of serum Vit D levels. The mean age of the patients was 36.36±10.43 years. The mean serum Vit D level (ng/mL) was 20.99±14.97 and 62.3% of patients (38/61) were having Vit D deficiency. The serum ALP levels (U/L) were significantly higher among Vit D deficient patients on student's t test analysis (Group 1: 244.64±106.93 vs Group 2: 187.46±70.00; t test value: -2.522, df: 58.565 and p value: 0.014). **Conclusions:** Although an inverse correlation was seen between serum Vit D and uric acid, the effect of Vit D deficiency in COVID-19 progression or disease severity is far yet to be assessed. The role of Vit D in the management of COVID-19 needs strong randomized control trial (RCT) evidence, but until then physicians should continue to treat deficiency and insufficiency of Vitamin D among COVID-19 patients, as there are hardly any adverse effects.

Keywords: CT value, Pearson's correlation test, Immunomodulator, D-dimer

Introduction

In December of the year 2019, an outbreak of pneumonia of unknown cause in Wuhan, China was reported to the World Health Organization (WHO) China Country Office. With the turn of the year (2020), increasing cases of this novel coronavirus were reported in different countries across the globe.

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The disease was later on named Coronavirus disease 2019 or COVID-19 and by March 2020, it was declared a pandemic by the WHO[1].

As of January 2021, a little over a year after the virus was first reported, the virus has infected at least 90 million people across the globe. Furthermore, it has taken 1.9 million lives worldwide[2]. With no known cure, medical practitioners all over the world were left with no treatment options, and while the vaccine were tried to be developed with efficacy and safety in a query, the virus continuously spread infecting and killing more and more people. Without a defined treatment protocol for COVID-19, medical practitioners were forced to find the best ways to manage the symptoms of infected patients and to prevent further complications. The curiosity of the scientific community was triggered by the lack of a cure and vaccine for COVID-19. While vaccine research is ongoing, scientific researchers began their search for a potential cure for COVID-19. One of the things that researchers investigated was the effect of Vitamin D

deficiency on the progression of COVID-19.While Vitamin D (Vit D) is commonly known for its contribution to bone health and metabolism of calcium and phosphorus, it has also been known to have an important function in the immune system[3,4]. Vitamin D was found to have a critical role as an "immunomodulator"[5]. Vitamin D modulates the body's immune response during infection[3].Vitamin D modulates both the innate immune system and the adaptive immune system by increasing the cathelicidins and B-defensins levels in the body, and by reducing the secretion of immunoglobulin by plasma cells and the production of pro-inflammatory cytokines, respectively[3,6]. It has both antimicrobial and anti- inflammatory effects and is known to be effective in preventing various upper respiratory tract infections (RTIs)[4,5]. It also has the potential to prevent or lessen the possibility of having complications from RTIs[6]. Vitamin D can also hasten the healing process of affected areas, specifically lung tissues[5].Vitamin D deficiency has been associated with increased levels of inflammatory cytokines and increased risk of pneumonia and viral upper respiratory tract infections[7]. It is also an important risk factor for acute respiratory distress syndrome (ARDS), which is an important determinant of the severity of illness among COVID-19 patients[7,8]. Vitamin D deficiency is also associated with increased episodes of thrombosis, which is commonly observed among COVID-19 patients[7].A low level of Vitamin D is commonly observed among those in the older age group, obese, and smokers, and among patients with chronic diseases like hypertension, gastroenterological disease, and diabetes[9]. It is also among these groups that COVID-19 was observed to be more prevalent and have more severe complications [6]. As per various literature sources, deficiency of Vitamin D is common in India, the Middle East, South America, Africa, and Australia[10-12].With the observation that the group of people with Vitamin D deficiency are also the same group suffering from more complications and higher mortality from COVID-19, it may be inferred that then Vitamin D deficiency might be an important risk factor for COVID-19. The patients with one of the fat malabsorption syndrome and bariatric patients are usually not able to absorb the fat-soluble vitamin, while patients with the nephritic syndrome also lose 25 (OH) D bound to the Vitamin D binding protein in the urine[10]. In subject with Vit D deficiency, its supplementation is able to reduce the risk to develop different viral infections[13]. Furthermore, subjects with low levels of Vit D at the time of COVID-19 testing were at higher risk to be positive for COVID-19 compared to those subjects with sufficient Vit D status[14].Throughout this pandemic, there has been a panoply of biochemical parameters suggested in the literature that are inextricably linked to the clinical progression of patients in different populations[15]. There have been a few published studies regarding the significance of Vit D levels as a predictor of disease severity, amongst other biomarkers like D-Dimer, CRP, and Ferritin in previous disease outbreaks like H1N1 and SARS[16-18]. Therefore, present study aimed to explore the association of Vitamin D3 with laboratory parameters including biochemical, inflammatory markers and CT Value of COVID-19 patients admitted to this hospital during pandemic. The result of this study can be used as a basis for stricter clinical trials to consequently determine whether Vitamin D supplementation would be a good part of clinical management for COVID-19.

Materials and methods

This cross-sectional study included 67 RT-PCR positive patients over a period of 3 months. To calculate the sample size for cross-sectional study, adequate literature for Vitamin D and COVID-19 in India was not available. Therefore, all RT-PCR positive patients were enrolled consecutively over a period of 3 months (May 2020 to August 2020). The blood samples were collected for RT-PCR positive patients during screening and during admission (new admissions) to the isolation ward of Government Institute of Medical Sciences (GIMS), Greater Noida to meet the required number of RT-PCR positive patients (67 patients). About 2ml of venous Blood was collected in EDTA vial for CBC estimation and 2 ml of blood was collected in citrate vial for doing D-dimer. For Biochemical analysis 3 ml of blood was collected in plain vial, before starting any kind of therapy. All tests were performed in an appropriate autoanalyzer after complying internal quality control. Similarly, the sample for Vitamin D3 was collected and the level of Vitamin D3 was estimated using chemiluminescence-based immunoassay analyser. Test results were received from the laboratory for the research purpose and all enrolled patients were provided with the reports. Those who were taking Vitamin D supplements or have taken them in the last 6 months were excluded from the study.

Statistical analysis

The data were collected and collated in Microsoft excel sheet. Data were presented as frequencies and percentages for qualitative and categorical variables. Mean with standard deviation or median with interquartile range was reported for continuous variables depending upon their distribution type. Appropriate statistical tests were applied for identifying significant statistical difference in the distribution of variables included in present study using SPSS (Statistical Package for Social Science) version 27.0.1.0. The correlation between Vit D and other parameters was assessed using Pearson correlation test. The patients were classified into two groups with Group 1 (Serum Vitamin D3 level < 20 ng/mL) and Group 2 (Vitamin D3 level $\geq$ 20 ng/mL). The comparison of quantitative variables (biochemical, inflammatory markers and CT Value) between two groups was performed using the Student t-test and by Chi-square test for categorical variables. All statistical analysis were carried out at 5% level of significance and p-value 0.05 was considered significant.

Ethical Consideration

All the patients were informed about the purpose of the study and written/digital consent was taken to participate in the study. The study protocol was approved by the institutional ethical committee of Government Institute of Medical Sciences, Greater Noida, Uttar Pradesh and the approval number for the study is GIMS IEC-ECR/1224/Inst./UP/2019. The study has been conducted as per the world medical association (WMA) Declaration of Helsinki for ethical principles for medical research involving human subjects.

Results

The present study enrolled 67 COVID-19 patients and among them only 61 patients had validated measurement of serum Vit D levels, so results are shown for those 61 patients only. Present study included 59.0% of males and 41.0% of females. The mean age of patients was 36.36±10.43 years, with age ranging between 19 to 67 years. 42.6% of patient belonged to 30-39 years of age group (Table 1).

Table 1. Baseline characteristics of COVID-19 patients.

Variable	Number/Mean	%/SD
Gender		
Female	25	41.0
Male	36	59.0
Age (in years)	36.36	10.43
Age group (in years)		
18-29	18	29.5
30-39	26	42.6
40-49	11	18.1
50-59	4	6.5
60 or above	2	3.3

Table 2. shows the normal distribution haematological findings among COVID-19 patients including Vit D with a mean of 20.99±14.97 ng/mL and value ranging from lowest 3.8 ng/mL to highest 76.2 ng/mL.

Table 2. Distribution of haematological findings among COVID-19 patients.

Lab parameters	Number	Minimum	Maximum	Mean	SD
CT Value	46	15.00	36.69	22.90	5.04
Total Bilirubin (mg/dL)	61	0.02	3.13	0.63	0.41
Direct Bilirubin (mg/dL)	61	0.012	1.830	0.37	0.25
Indirect bilirubin (mg/dL)	61	0.01	1.30	0.26	0.20
ALT (U/L)	61	6.6	275.9	51.14	47.27
AST (U/L)	61	11.6	190.8	48.06	41.71
ALP (U/L)	61	110.3	583.0	223.08	98.12
Total protein (g/dL)	61	5.38	8.32	7.05	0.65
Albumin (g/dL)	61	2.51	5.80	4.37	0.55
Creatinine (mg/dL)	61	0.52	3.89	1.14	0.45
Urea (mg/dL)	61	10.2	79.0	27.00	12.31
Uric acid (mg/dL)	61	1.1	13.9	5.59	2.51
Procalcitonin (ng/mL)	16	0.06	9.71	1.36	2.58
Ferritin (ng/mL)	53	9.75	1656.00	197.21	284.36
Vitamin D3 (ng/mL)	61	3.8	76.2	20.99	14.97
D-Dimer (mg/L)	37	0.10	6.39	1.67	2.08
LDH (U/L)	61	131.1	1332.0	352.78	187.88
CRP (mg/L)	60	0.2	157.2	20.74	37.85

In Figure 1.1 and 1.2 it is quite obvious that serum levels of Vit D (ng/mL) are inversely correlated with laboratory parameters such as Total Bilirubin (mg/dL), Direct Bilirubin (mg/dL), Indirect bilirubin (mg/dL), ALT (U/L), AST (U/L), ALP (U/L), Total protein (g/dL), Creatinine (mg/dL), Urea (mg/dL), Uric acid (mg/dL), Procalcitonin (ng/mL), Ferritin (ng/mL), D-Dimer (mg/L) and LDH (U/L), however Pearson correlation test showed that only Uric acid (mg/dL) level is significantly correlated (inversely, r = -0.267, p value = 0.037) with serum Vitamin D3 (ng/mL) levels (Table 3).

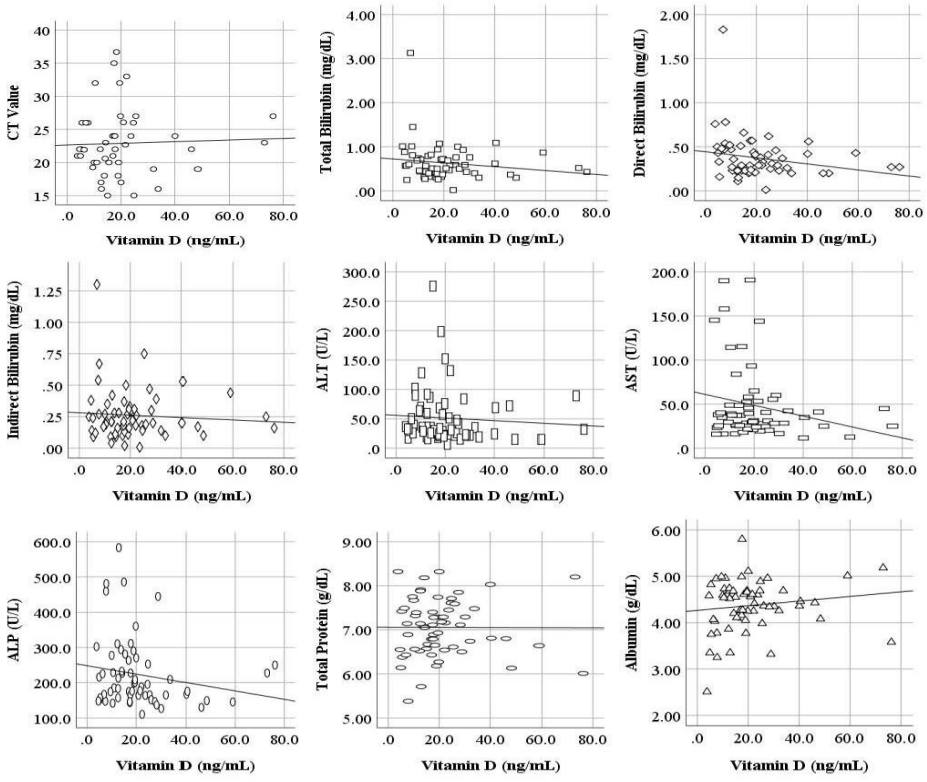

Fig 1:Scatter plot for correlation between Vitamin D and other laboratory parameters of COVID-19 patients.

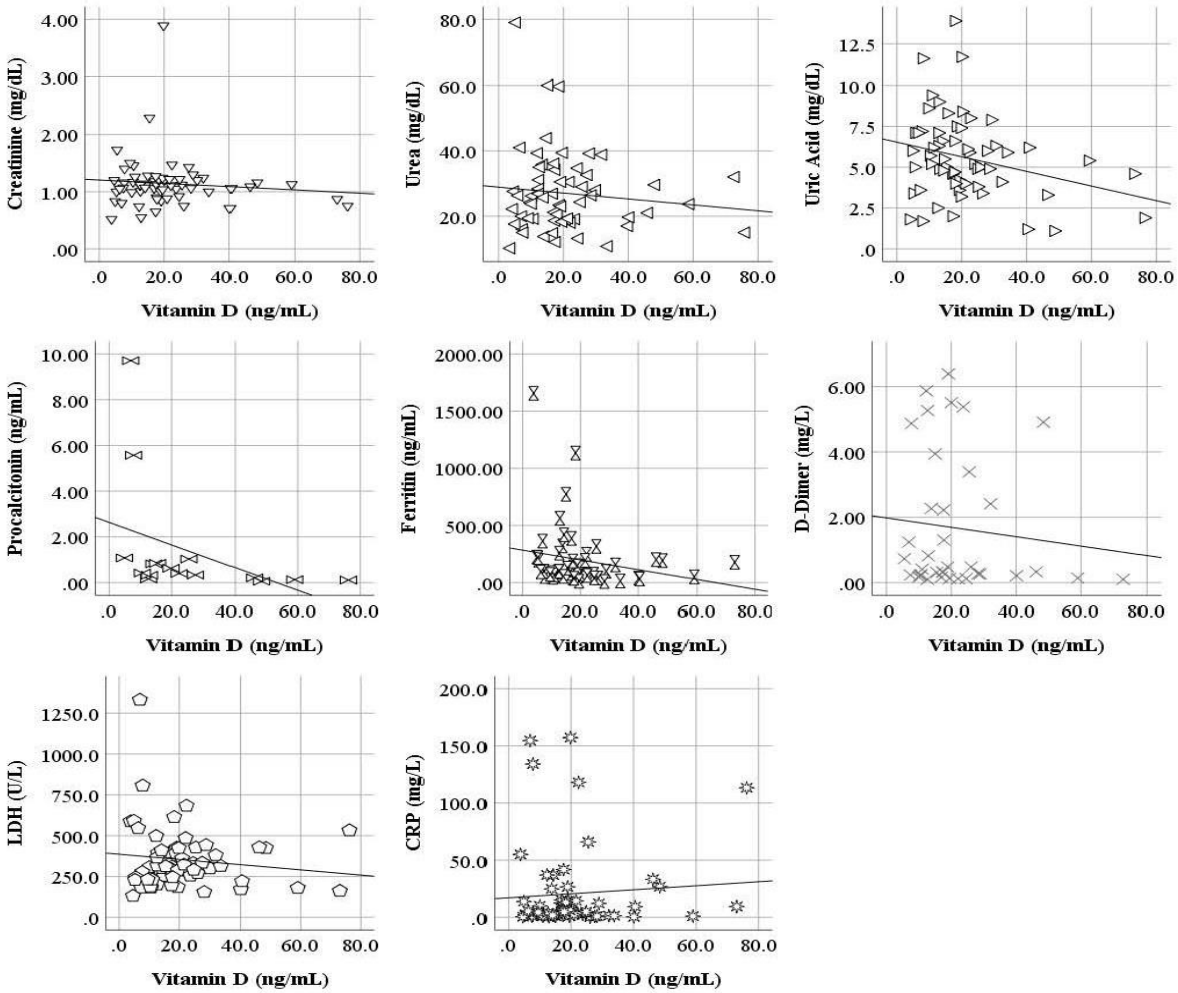

Fig 2:Scatter plot for correlation between Vitamin D3 and other laboratory parameters of COVID-19 patients.

Table 3. Correlation between Vitamin D and other laboratory parameters of COVID-19 patients.

Lab parameters	Vitamin D3 (ng/mL)		
	Number	Pearson Correlation	p value
Age (in years)	61	0.192	0.138
CT Value	46	0.038	0.801
Total Bilirubin (mg/dL)	61	-0.157	0.226
Direct Bilirubin (mg/dL)	61	-0.210	0.105
Indirect bilirubin (mg/dL)	61	-0.070	0.591
ALT (U/L)	61	-0.071	0.584
AST (U/L)	61	-0.221	0.087
ALP (U/L)	61	-0.182	0.161
Total protein (g/dL)	61	-0.004	0.975
Albumin (g/dL)	61	0.137	0.293
Creatinine (mg/dL)	61	-0.096	0.464
Urea (mg/dL)	61	-0.110	0.398
Uric acid (mg/dL)	61	-0.267	0.037*
Procalcitonin (ng/mL)	16	-0.399	0.126
Ferritin (ng/mL)	53	-0.206	0.139
D-Dimer (mg/L)	37	-0.102	0.547
LDH (U/L)	61	-0.127	0.329
CRP (mg/L)	60	0.070	0.596

*Statistically significant

On the basis of serum Vit D levels, the patients were classified in two groups, namely Group 1 (Vitamin D3 level < 20 ng/mL) and Group 2 (Serum Vitamin D3 level > 20 ng/mL). Present study revealed that 54.1% (38/61) of patients (Group 1) were having Vit D deficiency. The Chi square analysis for the gender of two groups showed that 66.7% (24/36) of male patients had Vit D3 deficiency whereas among females 56.0% (14/25) were deficient in Vit D3, but this difference was not significant (p value = 0.398).

Figure 2.1 and 2.2 shows the Boxplot for comparison of haematological findings among two groups of COVID-19 patients. The outliers (*) were observed for the Total Bilirubin (mg/dL), Direct Bilirubin (mg/dL), Indirect bilirubin (mg/dL), and LDH (U/L). The median score for laboratory parameter values of Direct Bilirubin (mg/dL), Indirect bilirubin (mg/dL), ALT (U/L), AST (U/L), ALP (U/L), Urea (mg/dL), Uric acid (mg/dL), Procalcitonin (ng/mL), Ferritin (ng/mL), D-Dimer (mg/L), LDH (U/L), and CRP (mg/L) was higher in Group 1, but Student T-test analysis (Table 4) showed that significant differences in the mean values for the two groups was observed only for ALP (Group 1: 244.64±106.93 U/L vs Group 2: 187.46±70.00 U/L, p value = 0.014) and Ferritin (Group 1: 246.06±341.01 ng/mL vs Group 2: 109.80±88.52 ng/mL, p value = 0.034).

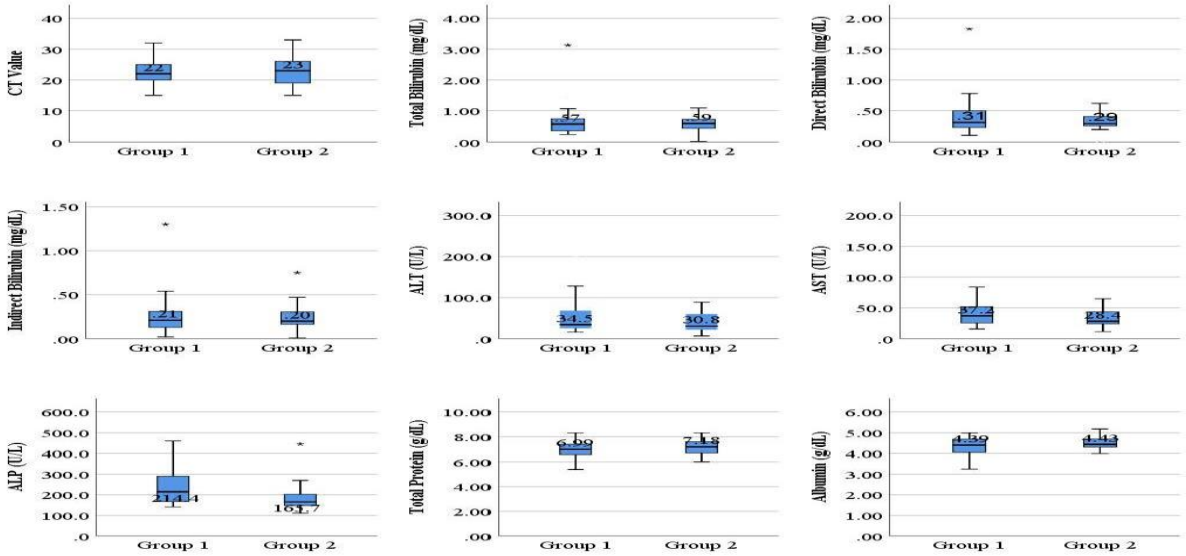

Fig 2:Boxplot for comparison of haematological findings among two groups of COVID-19 patients. (*Outliers)

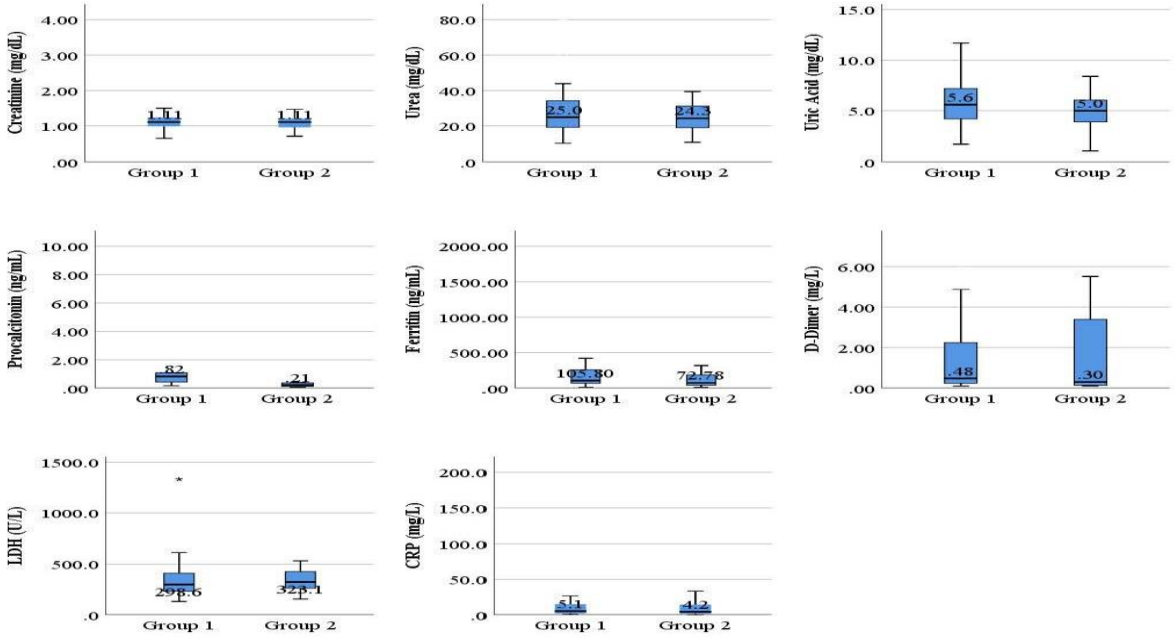

Fig 2: Boxplot for comparison of haematological findings among two groups of COVID-19 patients.

Table 4. Comparison of baseline characteristics/haematological findings among two groups of COVID-19 patients.

Lab parameters	Mean±SD		Test of significance
	Group 1	Group 2	t, df, p value
Age (in years)	36.03±10.89	36.65±9.86	0.225, 59, 0.823
CT Value	22.98±5.20	22.72±4.88	-0.166, 44, 0.869
Total Bilirubin (mg/dL)	0.66±0.49	0.66±0.493	0.704, 59, 0.484
Direct Bilirubin (mg/dL)	0.40±0.29	0.32±0.13	-1.178, 59, 0.244
Indirect Bilirubin (mg/dL)	0.26±0.22	0.26±0.16	0.031, 59, 0.975
ALT (U/L)	54.56±52.22	45.50±38.12	-0.723, 59, 0.473
AST (U/L)	54.10±47.68	38.08±27.46	-1.665, 59, 0.101
ALP (U/L)	244.64±106.93	187.46±70.00	-2.522, 59, 0.014*
Total protein (g/dL)	6.98±0.65	7.18±0.63	1.143, 59, 0.258
Albumin (g/dL)	4.32±0.60	4.46±0.45	0.953, 59, 0.345
Creatinine (mg/dL)	1.18±0.55	1.08±0.20	-0.801, 59, 0.426
Urea (mg/dL)	28.08±14.14	25.23±8.48	-0.874, 59, 0.386
Uric acid (mg/dL)	5.99±2.74	4.94±1.96	-1.616, 59, 0.111
Procalcitonin (ng/mL)	2.17±3.28	0.32±0.33	-1.679, 14, 0.131
Ferritin (ng/mL)	246.06±341.01	109.80±88.52	-2.201, 51, 0.034*
D-Dimer (mg/L)	1.65±2.08	1.69±2.17	0.050, 35, 0.960
LDH (U/L)	361.13±217.41	338.98±128.28	-0.443, 59, 0.659
CRP (mg/L)	21.42±40.04	19.57±34.61	-0.181, 58,0.857

*Statistically significant

Discussion

The present study compared, for the first time in India, Vit D serum levels with laboratory parameters including biochemical, inflammatory markers and CT Value of COVID-19 patients. Interestingly, patients with low Vit D serum levels had compromised biochemical and inflammatory markers findings. Vitamin D deficiency were common in COVID-19 affected patients as 54.1% of patients (38/61) had Vit D3 levels lower than 20 ng/mL. Male patients (66.7%) with had lower Vit D serum levels regardless of age as compared to females (56.0%).Vit D serum levels are the most accurate markers for defining Vit D state[25]. (OH)D is the major form of circulating of Vit D and it is the one measured. In general, in population Vit D levels lower than 20 ng/mL are commonly considered as deficiency status. Moreover, levels ranging 20 to 29 ng/mL should define insufficiency, while levels above 30 sufficiency [20]. Further , these levels are strictly related to Vit D effects on bone metabolism, while Vit D levels useful for investigation of other aspects of human health are under investigation[19,20]. For a long time, 10 ng/ml has been considered the Vit D level for defining deficiency[21,22]. Although the Endocrine Society has defined 20 ng/ml Vit D plasma levels, in terms of 25(OH)D, as the threshold to define the deficiency status there are insufficient evidences to clarify the optimal plasma Vit D concentration necessary for the global well-being[23,24].In present study Pearson correlation test showed that only Uric acid (mg/dL) level is significantly correlated (inversely, r = -0.267, p value = 0.037) with serum Vitamin D3 (ng/mL).

Vitamin D is also known to improve cellular immunity, partly by reducing the cytokine storm induced by the dysregulated innate immune system. This system generates both pro-inflammatory and anti-inflammatory cytokines in response to viral and bacterial infections and other chemical and oncological triggers[25]. Vitamin D may reduce the production of pro-inflammatory Th1 cytokines, such as tumor necrosis factor and interferon. Administering vitamin D may reduce the expression of pro-inflammatory cytokines and increase anti-inflammatory cytokines by macrophages like IL-10[26]. Vitamin D has known modulatory effects on adaptive immunity; 1,25(OH)2D3 suppresses responses mediated by the T helper cell type 1 (Th1) by mainly suppressing the production of inflammatory cytokines IL-2 and interferon-gamma (INF)[27,28]. Additionally, 1,25(OH)2D3 promotes cytokine production by the T helper type 2 (Th2) cells, promoting indirect suppression of Th1 cells by complementing this with actions mediated by a congregation of other cell lines[29]. Further- more, 1,25(OH)2D3 promotes induction of the T regulatory cells, resulting in suppression of inflammatory processes[30].

In present study Student T-test analysis showed that significant differences in the mean values for the two groups for various laboratory parameters was observed only for ALP (Group 1: 244.64±106.93 U/L vs Group 2: 187.46±70.00 U/L, p value = 0.014) and Ferritin (Group 1: 246.06±341.01 ng/mL vs Group 2: 109.80±88.52 ng/mL, p value = 0.034).A recent review and meta-analysis showed that Vitamin D supplementation taken daily or weekly, reduces ARI (acute respiratory infection) by 32% to 60%, thus having a protective effect and safe[31]. An increase in the number of clinical trials has been registered recently to understand the effect of Vitamin D intake on COVID-19 outcomes. Available clinical trials give a preliminary picture of the interaction between infected patients and Vitamin D intake. There is also speculation in the scientific community that recent COVID-19 publications lack rigorous peer-reviewing; therefore, caution must be taken in the interpretation of the published results. Another review concluded that a negative correlation between Vitamin D and COVID-19 was found among European countries, while retrospective studies found a correlation between Vitamin D and COVID-19 after adjusting the confounders[32]. The review suggested supplementation of Vitamin D to the susceptible group. However, consistency of Vitamin D deficiency has been observed in patients with severe forms of COVID-19. As mentioned above, Vitamin D intake plays a significant role in controlling the homeostasis of the human body thus providing enough support to hypothesize Vitamin D insufficiency may lead to adverse outcomes. Until there is a definitive result, it is worldwide recommended worldwide to ensure adequate intake of Vitamin D.

Conclusion

Although an inverse correlation between serum Vit D and uric acid, the effect of Vit D deficiency in COVID-19 progression or disease severity is far to be assessed. Our data underline a correlation between Vit D serum levels and different laboratory parameters. The role of Vit D in the management of COVID-19 needs strong RCT evidence, but until then physicians should continue to treat deficiency and insufficiency of Vitamin D among COVID-19, as there are hardly any adverse effects.

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