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Association of Vitamin D levels on the Clinical Outcomes of Patients Hospitalized for COVID-19 in a Tertiary Hospital

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Abstract

Objectives. This study aimed to compare the severity of COVID-19, inflammatory parameters and clinical outcomes among patients with normal and subnormal levels of Vitamin D.

Methodology. This is a retrospective cohort study of 135 patients admitted in a tertiary hospital for COVID-19. Patients were grouped according to their Vitamin D level. Primary outcome measure was the composite of all-cause mortality and morbidity. Other outcome measures determined were the comparison among the groups on the severity of COVID-19 infection, changes in inflammatory parameters, length of hospital stay and duration of respiratory support.

Results. There was a significant trend of higher ICU admission (p=0.024), mortality (p=0.006) and poor clinical outcome (p=0.009) among the Vitamin D deficient group. No significant difference was found for most of the inflammatory parameters, duration of hospital stay and respiratory support. Overall, patients with deficient, but not insufficient Vitamin D level had 6 times higher odds of composite poor outcome than those with normal Vitamin D (crude OR=5.18, p=0.003; adjusted OR=6.3, p=0.043).

Conclusion. The inverse relationship between Vitamin D level and poor composite outcome observed in our study suggests that low Vitamin D may be a risk factor for poor prognosis among patients admitted for COVID-19.

Key words: Vitamin D, Vitamin D deficiency, COVID-19

INTRODUCTION

Vitamin D deficiency is a public health problem affecting over a billion people among different age-groups worldwide.[1] Based on a 2013 National Nutrition Survey, Filipino adults have a high prevalence of Vitamin D insufficiency.[2] Vitamin D has a wide range of functions, not only in bone health but also in enhancement of the immune system. Vitamin D receptor (VDR) is present in most tissues including the immune system and calcitriol [$1,25(OH)_2D_3$], the active form of Vitamin D, can also be synthesized by antigen-presenting cells and lymphocytes where it inhibits the expression of inflammatory cytokines.[3,4] Several studies demonstrated the role of Vitamin D in reducing the risk of acute viral respiratory tract infections, including direct inhibition of viral replication or through anti-inflammatory or immunomodulatory functions.[4,5] Individuals with lower Vitamin D levels were more likely to have upper respiratory tract infection than those with adequate levels, even after adjusting for variables including season, age, gender, body mass index (BMI) and race.[4] During the COVID-19 pandemic, people with Vitamin D deficiency could be at higher risk of acquiring a more severe type of disease and Vitamin D supplementation has been hypothesized to be an effective means of preventing the worsening of disease course caused by SARS-CoV-2.[5-7]

It has been demonstrated that the human DPP-4/CD26 receptor, a virulence factor in COVID-19 infection, can be reduced by adequate levels of vitamin D.[6] The bioavailability and expression of angiotensin-converting enzyme 2 (ACE2), which is responsible for inactivating the virus, are both enhanced by Vitamin D.[8] Acute lung injury and other organ failures in COVID-19 are caused by activation of the renin-angiotensin-aldosterone system (RAS), and Vitamin D can act as a negative RAS regulator and inducer of ACE2 to mitigate the effects of the virus.[8] Upon correction of the Vitamin D deficiency, the immunological changes could begin within a short period, as immune responses are dynamic processes that protect against viral infections.[4] However, data is still conflicting on whether Vitamin D supplementation can be used as prophylaxis or adjunctive therapy in COVID-19.

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To date, there is no local data regarding the association of Vitamin D levels with the severity of COVID-19 infection. This study aimed to compare the severity of COVID-19, inflammatory parameters and clinical outcomes among patients with normal and subnormal levels of Vitamin D, and to determine the association of Vitamin D level with the all-cause mortality and morbidity among patients admitted due to COVID-19.

METHODOLOGY

This is a retrospective study that utilized chart review of COVID-19 patients admitted at Chinese General Hospital and Medical Center from January 2021 to August 2021, which were managed by or referred to Endocrinology service. Included patients were at least 18 years old, confirmed positive for COVID-19 by RT-PCR and with Vitamin D assay [25(OH)D] done upon admission or within 2 weeks from date of admission. Exclusion criteria include pregnant patients, those with granulomatous disorders, on medications that may affect Vitamin D metabolism (chronic glucocorticoid use, antiseizure medications, antiretroviral medication such as efavirenz and zidovudine), malabsorption syndromes, hyperparathyroidism and chronic kidney disease (CKD) on dialysis.

Patients were categorized according to 25(OH)D levels into normal (≥30 ng/ml; SI: >75 nmol/L), insufficient (21–29 ng/ml; SI:50-75 nmol/L) or deficient (<20 ng/ml; SI: <50 nmol/L) group as defined by the Endocrine Society Guidelines.[9] Primary outcome investigated was the composite of all-cause mortality and morbidity which included respiratory decompensation (the need for invasive or increase in requirement of noninvasive ventilation), cardiac decompensation (the need for vasopressors or episodes of pulseless rhythms) or acute kidney failure with the need for renal replacement therapy and ICU admission. Other outcomes investigated include changes in inflammatory parameters [ferritin, procalcitonin PCT), C-reactive protein (CRP), lactate dehydrogenase (LDH) and D-dimer], length of stay of hospitalization (LOS) and duration of respiratory support.

Laboratory tests for 25(OH)D, ferritin and PCT were measured with Abbott Architect machine, while LDH and CRP with Beckman Coulter using the principle of chemiluminescence immunoassay; D-dimer was measured with STA Compact Max using the principle of photometry. Comparison was made between the groups in terms of severity of COVID-19 infection and changes in inflammatory parameters (ferritin, PCT, CRP, LDH, D-dimer). Severity of COVID-19 was defined using the World Health Organization (WHO) Classification used in the clinical management of COVID-19 Interim Guidance 2020[10] (Supplemental Table B).

Sample size

We estimated the sample size of at least 83 patients for the study population. This was based on 80% statistical power, 95% confidence level and 94.3% prevalence of Vitamin D deficiency among COVID-19 patients based on a study by Demir et al.[11] The current study included all admitted COVID-19 patients with Vitamin D assay that were referred to or managed by the Section of endocrinology for the specified time.

Data analysis

All data were encoded in MS Excel and Stata MP version 16 software was used for data processing and analysis. Continuous variables were presented as mean (standard deviation/SD) or median (interquartile range/IQR) depending on the data distribution. Categorical variables were presented as frequencies and percentages. One-way ANOVA or Kruskal Wallis test was performed to compare continuous variables. Significant Kruskal Wallis test was further analyzed using Dunn's test. Chi-square test or Fisher's exact test was used to analyze categorical variables.

In order to determine the association between Vitamin D level and composite poor outcome, logistic regression analysis with Firth's bias correction was done. Screening for potential confounders was performed using simple logistic regression analysis and a cutoff of $p<0.20$[12] (Supplemental Table A). Model building was performed using multiple logistic regression analysis and significant confounders were retained in the model using the change-in-estimate criterion of 10%. Imputation for missing data was not performed. $P \leq 0.05$ were considered statistically significant.

Ethical Considerations

This study adhered to the ethical considerations and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012 and National Ethics Guidelines for Health Research 2017. The study commenced upon the approval of the Chinese General Hospital and Medical Center Research Ethics Review Board (CGHMC RERB2021-F-52). The results and patient information were kept strictly confidential by the primary investigator. All data were encoded using a password-protected spreadsheet.

RESULTS

A total of 137 charts were retrieved. Two patients were excluded due to the use of antiseizure medications and comorbidity of CKD on dialysis. Among 135 patients analyzed, the median Vitamin D level was 25ng/ml (SI:50nmol/L) [IQR: 19.8-31; Range: 6.7-73]. Only 38 (28.2%) patients had normal Vitamin D level. Of those with abnormal result, 63 (46.7%) had insufficiency, and 34 (25.2%) had deficiency.

Table 1 shows the baseline characteristics of patients including the comorbidities. Most patients were 60-75 years old, male and had moderate COVID-19 infection.

Majority had normal BMI for each group (*Normal=84.2%, Insufficient=76.2%, Deficient=44.1%*). However, there was a higher percentage of overweight (29.4%) and obesity (23.5%) among the Vitamin D deficient group. BMI (p=0.0005), type 2 diabetes mellitus (T2DM) (p=0.013), heart failure (p=0.036) and COVID severity (p=0.045) (Figure 1) significantly differ by Vitamin D level. In terms of severity of infection, more patients had severe and critical illness with hypovitaminosis D compared to normal Vitamin D group (p=0.045) (Figure 1).

For the baseline inflammatory markers, only median CRP was significantly different by Vitamin D level. Further analysis using Dunn's test revealed that median CRP of patients with deficient Vitamin D was significantly higher compared to normal (p=0.0070) and insufficient (p=0.0111)

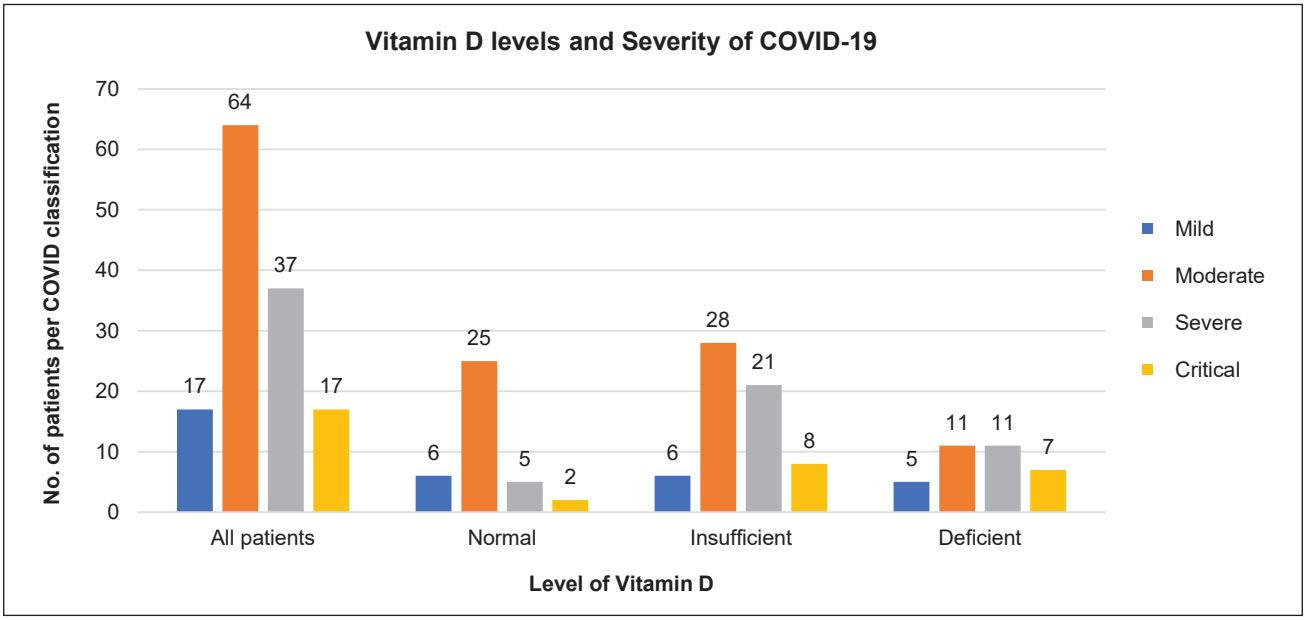

Figure 1. Severity of COVID-19 infection in relation to levels of Vitamin D. In terms of severity of the disease, more patients have severe and critical illness with hypovitaminosis D compared to normal Vitamin D group (p=0.045).

Table 1. Baseline demographic and clinical characteristics by Vitamin D level (n=135)

	All patients (n=135) n (%)	Normal Vitamin D >30 ng/ml (n=38) n (%)	Insufficient Vitamin D 21-29 ng/ml (n=63) n (%)	Deficient Vitamin D <20 ng/ml (n=34) n (%)	*p*
Age (in years), mean	64.8 ± 14.0	67.8 ± 12.0	62.8 ± 14.7	65.3 ± 14.4	0.2102[a]
18-39 years old	6 (4.4)	0 (0.0)	4 (6.4)	2 (5.9)	0.684[b]
40-59 years old	39 (28.9)	10 (26.3)	21 (33.3)	8 (23.5)	
60-75 years old	62 (45.9)	19 (50.0)	26 (41.3)	17 (50.0)	
Above 75 years old	28 (20.7)	9 (23.7)	12 (19.0)	7 (20.6)	
Sex					
Female	50 (37.0)	16 (42.1)	25 (39.7)	9 (26.5)	0.327[c]
Male	85 (63.0)	22 (57.9)	38 (60.3)	25 (73.5)	
Asia-Pacific BMI (in kg/m^2), median	23 [IQR: 21-26]	22 [IQR: 20-23]	22 [IQR: 21-25]	26.4 [IQR: 22-30]	0.0005*[d]
Underweight (18.5)	2 (1.5)	1 (2.6)	0	1 (2.9)	0.001*[b]
Normal (18.5-22.9)	95 (70.4)	32 (84.2)	48 (76.2)	15 (44.1)	
Overweight (23-24.9)	25 (18.5)	5 (13.2)	10 (15.9)	10 (29.4)	
Obese (≥25)	13 (9.6)	0	5 (7.9)	8 (23.5)	
Co-morbid condition, % with					
Hypertension	82 (60.7)	26 (68.4)	38 (60.3)	18 (52.9)	0.404[c]
Diabetes Mellitus	125 (92.6)	38 (100.0)	54 (85.7)	33 (97.1)	0.013*[b]
Cerebrovascular Disease	8 (5.9)	2 (5.3)	5 (7.9)	1 (2.9)	0.732[b]
Ischemic Heart Disease	10 (7.4)	5 (13.2)	4 (6.4)	1 (2.9)	0.243[b]
Heart Failure	3 (2)	3 (7.9)	0 (0.0)	0 (0.0)	0.036*[b]
Chronic Kidney Disease (not on Dialysis)	14 (10.4)	3 (7.9)	6 (9.5)	5 (14.7)	0.628[b]
Chronic Lung Disease (Asthma, COPD)	2 (1.5)	1 (2.6)	0 (0.0)	1 (2.9)	0.283[b]
Thyroid Diseases	13 (9.6)	3 (7.9)	7 (11.1)	3 (8.9)	0.931[b]
Malignancy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Tuberculosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Chronic Liver Disease	3 (2)	1 (2.6)	2 (3.2)	0 (0.0)	0.797[b]
Others	3 (2)	1 (2.6)	1 (1.6)	1 (2.9)	1.000[b]

[a]One Way ANOVA was used; [b]Fisher's exact test was used; [c]Chi-square test was used; [d]Kruskal-Wallis test was used. Significant results were further analyzed using Dunn's test

group. No significant difference in median CRP was found between normal and insufficient Vitamin D (p=0.3317). Baseline and final results for the inflammatory markers were obtained and the median of these changes were compared among the 3 groups as shown in Table 2. Only changes in D-dimer and CRP significantly differ by Vitamin D level. Further analysis using Dunn's test showed that the median change (i.e., decrease) in D-dimer of patients with normal Vitamin D was significantly higher compared to insufficient Vitamin D level (p=0.0032), but not with deficient group (p=0.1542). Only 65.4% of patients with deficient Vitamin D had a decrease in CRP compared to 89.7% of normal Vitamin D level, and 86% of insufficient Vitamin D.

Overall incidence of poor clinical outcome was 34.8% (95% CI: 27.2-43.3% p=0.009). Clinical outcome was significantly different by Vitamin D level, such that more than half of patients with deficient Vitamin D had poor outcome compared to only 18.4% of those with normal Vitamin D (Table 3). Of the specific poor outcomes of interest, respiratory decompensation and initiation of renal replacement were not significantly different across the 3 groups. Compared to those with normal Vitamin D levels, a higher proportion of insufficient and deficient Vitamin D levels required vasopressor or inotropic support. None of the patients in the normal and insufficient Vitamin D groups had episodes of pulseless electrical activity or

Table 2. Change in inflammatory parameters by Vitamin D level

	All patients (n=135) Median [IQR]	Normal Vitamin D >30 ng/ml (n=38) Median [IQR]	Insufficient Vitamin D 21-29 ng/ml (n=63) Median [IQR]	Deficient Vitamin D <20 ng/ml (n=34) Median [IQR]	*p*
Ferritin (ng/mL) (n=131)	287.0 [IQR: 25.0-851.0]	341.0 [IQR: 86.0-823.0]	292.5 [IQR: 15.0-1164.0]	230 [IQR: -238-851]	0.8545[a]
Increased	5 (13.5)	5 (13.5)	12 (20.0)	9 (26.5)	0.392[b]
Decreased	32 (86.5)	32 (86.5)	48 (80.0)	25 (73.5)	
LDH (U/L) (n=124)	209.5 [IQR: 48.5-392.5]	298.5 [IQR: 120.0-532.0]	149.0 [IQR: 31.0-277.0]	198.0 [IQR: -7.0-285.0]	0.0535[a]
Increased	27 (21.8)	5 (13.9)	13 (23.6)	9 (27.3)	0.366[b]
Decreased	24 (78.2)	31 (86.1)	42 (76.4)	24 (72.7)	
D-dimer (ug/ml) (n=122)	0.1 [IQR: -0.3-2.0]	0.3 [IQR: 0.1-0.9]	0 [IQR: -0.6-0.4]	0.2 [IQR: -0.9-0.9]	0.0223*[a]
Increased	41 (33.6)	5 (13.5)	26 (46.4)	10 (34.5)	0.004*[c]
Decreased	80 (65.6)	32 (86.5)	29 (51.8)	19 (65.5)	
No change	1 (0.8)	0	1 (1.8)	0	
Procalcitonin (ng/ml) (n=106)	0.1 [IQR: 0.0-0.3]	0.1 [IQR: 0.0-0.4]	0.1 [IQR: 0.0-0.2]	0.1 [IQR: -0.1-0.3]	0.7489[a]
Increased	21 (19.8)	3 (10.7)	10 (20.0)	8 (28.6)	0.333[c]
Decreased	83 (78.3)	25 (89.3)	38 (76.0)	20 (71.4)	
No change	2 (1.9)	0	2 (4.0)	0	
C-reactive Protein (mg/L) (n=98)	3.5 [IQR: 1.2-5.5]	3.5 [IQR: 1.8-6.4]	3.7 [IQR: 1.4-5.5]	1.4 [IQR: 0-4.7]	0.1687[a]
Increased	14 (14.3)	3 (10.3)	6 (14.0)	5 (19.2)	0.025*[c]
Decreased	80 (81.6)	26 (89.7)	37 (86.0)	17 (65.4)	
No change	4 (4.1)	0	0	4 (15.4)	

[a]Kruskal-Wallis test was used. Significant results were further analyzed using Dunn's test; [b]Chi-square test was used; [c]Fisher's exact test was used

Table 3. Clinical outcomes by Vitamin D level (n=135)

	All patients (n=135) n (%)	Normal Vitamin D >30 ng/ml (n=38) n (%)	Insufficient Vitamin D 21-29 ng/ml (n=63) n (%)	Deficient Vitamin D <20 ng/ml (n=34) n (%)	*p*
Poor clinical outcome	47 (34.8)	7 (18.4)	22 (34.9)	18 (52.9)	0.009*[a]
Respiratory decompensation	38 (28.2)	7 (18.4)	18 (28.6)	13 (38.2)	0.174[a]
Requirement for vasopressor and/or inotropic support	28 (20.7)	2 (5.3)	13 (20.6)	13 (38.2)	0.003*[a]
Ventricular tachycardia or fibrillation/Pulseless Electrical Activity/ Resuscitated cardiac arrest	3 (2.2)	0 (0.0)	0 (0.0)	3 (8.8)	0.015*[b]
Initiation of renal replacement therapy	7 (5.2)	0	4 (6.4)	3 (8.8)	0.217[b]
Death from any cause	20 (14.8)	1 (2.6)	9 (14.3)	10 (29.4)	0.006*[a]
Length of hospital stay, all patients	12.0 [IQR: 9.0-17.0]	11.5 [IQR: 9.0-15.0]	12.0 [IQR: 9.0-17.0]	13.0 [IQR: 8.0-19.0]	0.7424[c]
Length of hospital stay, survivors (n=115)	11.0 [IQR:9.0-16.0]	11.0 [IQR: 9.0-15.0]	11.0 [IQR: 9.0-16.0]	12.0 [IQR: 7.0-18.5]	0.9488[c]
ICU admission					
No	112 (83.0)	36 (94.7)	52 (82.5)	24 (70.6)	0.024*[a]
Yes	23 (17.0)	2 (5.3)	11 (17.5)	10 (29.4)	
Length of ICU stay, median (IQR) (n=23)	14 [IQR: 13-22]	11 [IQR: 8-14]	17 [IQR: 14-30]	16 [IQR: 13-21]	0.3558[c]
Respiratory support (n=134)					
No	9 (6.7)	3 (7.9)	4 (6.4)	2 (6.1)	1.000[b]
Yes	125 (93.3)	35 (92.1)	59 (93.6)	31 (93.9)	
Length on respiratory support, median (IQR) (n=125)	10 [IQR: 7-14]	8 [IQR: 6-10]	10 [IQR: 7-14]	13 [IQR: 6-17]	0.0550[c]
Non-invasive					
Nasal cannula (n=64)	7 [IQR: 6-10]	7 [IQR: 6-9]	8 [IQR: 6.5-10]	6 [IQR: 6-8]	0.3159[c]
Face mask (n=7)	10 [IQR: 7-13]	13 [IQR: 10-16]	8 [IQR: 7-9.5]	13 [IQR: 13-13]	0.1503[c]
HFNC (n=36)	14 [IQR: 11-21]	10 [IQR: 7-15]	13.5 [IQR: 11-22]	14 [IQR: 14-25]	0.1570[c]
Invasive					
Mechanical Ventilation (n=18)	16.5 [IQR: 13-21]	20 [IQR: 20-20]	17 [IQR: 14-24]	15 [IQR: 13-21]	0.8371[c]

[a]Chi square test was used; [b]Fisher's exact test was used; [c]Kruskal-Wallis test was used

Table 4. Association between Vitamin D level and composite poor outcome (n=107)

	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)[a]	*p*
Vitamin D level				
Normal (> 30 ng/ml)	Ref	Ref	Ref	Ref
Insufficient (21-29 ng/ml)	2.326 (0.858-6.307)	0.097	2.634 (0.566-12.261)	0.217
Deficient (<20 ng/ml)	5.174 (1.717-15.588)	0.003*	6.331 (1.059-37.836)	0.043*

Ref: Reference category; [a]Adjusted for the significant confounding effects of age, COVID-19 severity, hypertension, ischemic heart disease, chronic lung disease, baseline D-dimer, baseline LDH, and baseline procalcitonin

resuscitated cardiac arrest but this outcome was observed in 8.8% of Vitamin D deficient patients.

Overall mortality for the study population was 14.8%. There was a significant trend of higher mortality for the deficient group (p=0.006), while only 1 patient with normal Vitamin D died. There was no significant difference with the duration of hospitalization and the median LOS among the survivors was 11 days, (range of 5-45 days). Twenty-three patients (17%) in this study were admitted to the ICU. The proportion significantly differ by Vitamin D level such that only 5.3% of those with normal Vitamin D were admitted to ICU compared to 17.5% of insufficient and 29.4% of deficient levels. The type of respiratory support used and the length of respiratory support were not significantly different by Vitamin D level.

For the determination of the association between Vitamin D level and composite poor outcome, only 107 patients with complete data for all variables of interest were included. Vitamin D deficiency was significantly associated with composite poor outcome even after controlling for significant confounders (Table 4). Patients with deficient Vitamin D level had about 5 times higher odds of poor outcome than those with normal Vitamin D level (OR=5.177). After controlling for the effects of confounders, patients with deficient vitamin D had 6 times higher odds of poor outcome than patients with normal Vitamin D levels (adjusted OR = 6.3).

DISCUSSION

This is the first study in the Philippines to have investigated the association of Vitamin D levels with the clinical outcomes of COVID-19 infection. Majority of the subjects in our study had T2DM, which is the most common reason for COVID-19 referrals to the endocrinology service. Most of the baseline characteristics were comparable among the groups except for T2DM, obesity and heart failure; however these findings may not reflect data in the general population. Vitamin D deficiency has been shown to modify insulin synthesis and secretion, not only through regulation of plasma calcium, but also through a direct action on pancreatic beta-cell function, suggesting its pathogenicity for T2DM.[13] High prevalence of Vitamin D deficiency in subjects with above normal BMI is a well-documented finding. Causes include volumetric dilution into the greater volumes of fat, serum, liver or muscles, impaired hepatic 25-hydroxylation and possibly due to sequestration and altered metabolism in adipose tissues.[14] Hypovitaminosis D is also implicated in numerous cardiovascular diseases.[15] However, there was limited number of patients with documented ischemic heart disease or heart failure in our study.

In our results, there were more patients with deficient Vitamin D among the critical group and subnormal 25(OH) D levels for the severe group. This finding is similar to a prospective study by Campi et al., in which low 25(OH)D levels at hospital admission were associated with increased interleukin-6 (IL-6) levels and predicted both the disease severity and mortality during the course of hospitalization, independently of other comorbidities. They even suggested that 25(OH)D can be a considered as a useful prognostic marker for COVID- 19.[16] This study also supports the meta-analysis by Pereira et al., that severe type of cases had more individuals with deficient Vitamin D levels compared to mild cases, especially among the elderly.[17]

Vitamin D enhances cellular innate immunity partly through the induction of antimicrobial peptides, including human cathelicidins and defensins, thereby reducing the cytokine storm and severity of infection. Administering Vitamin D reduces the expression of pro-inflammatory cytokines and increases the expression of anti-inflammatory cytokines.[9] Human DPP-4/CD26, the target of the S1 domain of the COVID-19 spike glycoprotein, has been suggested as a virulence factor in COVID-19 infection. The expression of the DPP-4/CD26 is reduced significantly in vivo upon the correction of Vitamin D insufficiency and there is evidence that Vitamin D may reduce some of the negative immunological consequences.[8]

According to some studies, serum concentrations of 25(OH)D were inversely associated with pro-inflammatory cytokines, IL-6, CRP and the risk of pneumonia, acute respiratory distress syndrome (ARDS), diabetes and heart failure.[18,19] Biomarkers have been used for confirming and classifying disease severity in COVID-19 and according to some investigators, CRP was elevated in 60.7% of patients, PCT in 5.5%, and LDH in 41% of patients.[19] Patients with severe COVID-19 have a higher incidence of elevated CRP than those with a mild form of disease. Although CRP is a nonspecific marker, it becomes more specific to the bioactivity of IL-6 and formation of a cytokine storm in patients with severe COVID-19. Analysis of Vitamin D status and CRP levels among affected patients suggests that subjects with Vitamin D deficiency have more incidence of elevated CRP and cytokine storm than patients with normal Vitamin D status.[20]

In this study, only the baseline CRP and downward trend of CRP and D-dimer levels showed a significant difference between the 3 groups. D-dimer elevation signify a hyper-fibrinolysis state and increased inflammatory burden, worsening respiratory problems induced by COVID-19 infection. Significantly higher levels are found in those with critical illness and is used as a prognostic marker for in-hospital mortality or severity of COVID-19 infection.[21,22] Vitamin D metabolites have the ability to regulate different pro- and anti-thrombotic agent of coagulation cascade and some authors proposed that Vitamin D supplementation along with prophylactic anticoagulant can be potentially useful for COVID-19 associated coagulopathy.[23]

Studies on ferritin levels in COVID-19 patients have yielded equivocal results. Two retrospective studies have reported a minimal role of ferritin in predicting ICU admission and need for ventilation and failed to predict mortality.[19] Another study showed findings to the contrary, that ferritin levels could predict severe disease and mortality.[18] A small retrospective study revealed that ferritin was the last parameter to return to normal making it a marker of severity and less useful for monitoring the course of disease.[24] In our investigation, the insignificant baseline levels of ferritin, LDH and procalcitonin may reflect an earlier course of their disease, while the insignificant decrease in markers from the initial results could be due to coexisting infections or comorbidities and that other repeated levels of inflammatory parameters (between baseline and final values) were not taken into account during the course of their hospitalization.

Increased generation of pro-inflammatory cytokines in COVID-19 are responsible for the development of ARDS. The SARS-CoV-2 does not only affect the respiratory system but can also cause acute myocardial or kidney injury. In this study, among the specific clinical outcomes (Table 3), there was a higher trend of cardiac decompensation among the hypovitaminosis D groups. VDR is particularly prevalent in vascular smooth muscle and myocardium. Vitamin D, as an anti-inflammatory, can modulate nitric oxide (NO) production and inhibit endothelial protein expression for leukocyte adhesion; while as an antithrombotic, plays a role in the upregulation of thrombomodulin. Vitamin D also has an anti-remodeling effect, it decreases pro-inflammatory cytokines, reducing the fatality risk of obesity and heart failure among COVID-19 patients.[25]

Countries with below average Vitamin D levels have higher numbers of COVID-19 cases and mortality,[26] and hospital LOS was significantly shorter in the sufficient group than in the deficient group suggesting that treatment of Vitamin D deficiency can prevent COVID-19 severity or death.[17,20] In another prospective study, among hospitalized patients with moderate to severe COVID-19, those with severe 25(OH)D deficiency (<10 ng/mL) exhibited a trend for longer hospital LOS, although not statistically significant, compared with patients with higher 25(OH)D concentrations.[27] Other observational studies also lack significant association of hypovitaminosis D with ICU admission, type and length of respiratory support, need for mechanical ventilation and mortality.[28,29] Conflicting results may be due to a smaller sample size in some studies, presence of bacterial co-infections and differences in study design and cut-off for categorizing Vitamin D level. Our investigation did not reveal statistically significant difference among the type and length of respiratory support and LOS. These could be due to smaller sample size and that majority of the subjects had moderate COVID-19. Medications used for COVID-19 and different patterns of weaning from respiratory support by various infectious disease specialists and pulmonologists could have also affected the results.

Overall, congruent with other studies, our research showed that serum 25(OH)D levels were significantly associated with risk of ICU admission, COVID-19-related in-hospital mortality and with the composite poor outcome. Furthermore, it was found to be associated with CRP and D-dimer which are among the most useful predictive biomarkers for poor prognosis of COVID-19.[6,23,27,30] These findings suggest the role of Vitamin D in suppressing some of the inflammatory markers in cytokine storm that are responsible for the poor clinical outcome or mortality among COVID-19 patients. Whether Vitamin D supplementation as a preventive means in the general population is still debatable. In the CORONAVIT trial, there was no statistically significant difference in the incidence or severity of acute COVID-19 infection between the subjects treated with Vitamin D and those without supplementation for both intention-to-treat and sensitivity analysis.[31] In the recent Philippine COVID-19 clinical practice guidelines, there is also insufficient evidence based from randomized clinical trials in other countries, on the use of Vitamin D supplementation as an adjunct therapy.[32]

Limitations and recommendations

There were some limitations in this study. Not all the inflammatory parameters requested during the entire hospitalization were taken into account, including the dates of final results from the baseline values in the analysis. Medications that were used for treatment of COVID-19 may also had an effect in the clinical outcomes. Another limitation is the possibility of information bias as the investigators relied on electronic medical records and clinical notes written on charts to gather the necessary data. Due to its retrospective nature, some records had missing data hence studies with larger sample size are also recommended for higher statistical power. The analysis for composite poor outcome may also overestimate the risk due to the small sample size. Confounding bias was addressed using regression analysis, although there may be other factors that could have influenced poor outcome that was not measured in this study due to unavailability in medical charts (residual confounding). The external validity may not also be generalizable to the current population since characteristics of the subjects in this study may be different from other institutions, especially in government

hospitals. Yet these observations, together with the relative safety of Vitamin D supplementation, further support the need for prospective or well-designed intervention trials aimed at exploring whether Vitamin D supplementation and correction of Vitamin D deficiency might prevent the risk of morbidity and mortality in patients with COVID-19 infection.

CONCLUSION

The high occurrence of hypovitaminosis D in severe and critical COVID-19 patients implies a potential relation to poor prognosis. Overall, patients with deficient, but not insufficient Vitamin D level had 6 times higher odds of composite poor outcome than those with normal Vitamin D after adjusting for potential confounders (Crude OR = 5.18; adjusted OR = 6.3). The inverse association between serum 25(OH)D levels and composite poor outcome (ICU admission, in-hospital mortality and morbidity) observed in our retrospective study suggests that a lower Vitamin D status upon admission may be an independent risk factor for poor prognosis in COVID-19.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
MKAT: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **RA:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing review and editing, Supervision; **KL:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing review and editing, Supervision.

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SUPPLEMENTARY TABLES

Supplementary Table A. Screening for potential confounders: Univariable and multivariable logistic regression analysis of factors associated with Vitamin D level

	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Age (in years)	1.045 (1.012-1.078)	0.006*	1.044 (0.994-1.097)	0.084
Sex				
Female	Ref	Ref	Ref	Ref
Male	1.313 (0.589-2.929)	0.505	Removed	Removed
Weight (in kg)	0.982 (0.944-1.020)	0.344	Removed	Removed
BMI (in kg/m^2)	0.952 (0.866-1.047)	0.309	Removed	Removed
COVID-19 severity				
Mild	Ref	Ref	Ref	Ref
Moderate	3.202 (0.168-61.009)	0.439	4.092 (0.182-92.043)	0.375
Severe/ Critical	60.304 (3.252-1118.15)	0.006*	42.60 (1.835-988.65)	0.019*
Co-morbid condition, % with				
Hypertension	2.072 (0.901-4.768)	0.087	2.648 (0.748-9.381)	0.131
Diabetes Mellitus	0.500 (0.117-2.136)	0.349	Removed	Removed
Cerebrovascular Disease	2.550 (0.324-20.043)	0.373	Removed	Removed
Ischemic Heart Disease	3.611 (0.952-13.696)	0.059	6.428 (0.797-51.845)	0.081
Heart Failure	2.550 (0.324-20.043)	0.373	Removed	Removed
Chronic Kidney Disease (not on Dialysis/non-HD requiring)	2.763 (0.800-9.535)	0.108	Removed	Removed
Chronic Lung Disease (Asthma, COPD)	7.771 (0.364-166.956)	0.189	5.446 (0.146-203.83)	0.359
Thyroid Diseases	1.56 (0.488-4.986)	0.453	Removed	Removed
Chronic Liver Disease	1.494 (0.150-14.847)	0.732	Removed	Removed
Complete blood count, baseline				
WBC (x10^9)	1.050 (0.972-1.133)	0.216	Removed	Removed
Neutrophil count (%)	0.986 (0.953-1.021)	0.441	Removed	Removed
Lymphocyte count (%)	1.014 (0.981-1.049)	0.406	Removed	Removed
Hemoglobin (g/L)	1.003 (0.980-1.026)	0.814	Removed	Removed
Platelet (x10^9)	1.00 (0.995-1.002)	0.531	Removed	Removed
Electrolytes, baseline				
Sodium (mmol/L)	0.991 (0.937-1.048)	0.756	Removed	Removed
Potassium (mmol/L)	1.276 (0.605-2.692)	0.523	Removed	Removed
Creatinine (mmol/L)	1.008 (1.000-1.016)	0.064	Removed	Removed
Inflammatory markers, baseline				
Ferritin (ng/mL)	1.000 (1.000-1.000)	0.096	Removed	Removed
LDH ((U/L))	1.001 (1.000-1.002)	0.014*	1.001 (0.999-1.002)	0.437
D-dimer (ng/ml)	1.295 (1.029-1.629)	0.027*	1.058 (0.830-1.349)	0.648
Procalcitonin (ng/ml)	1.712 (0.978-2.997)	0.060	1.224 (0.442-3.384)	0.697
C-reactive Protein (mg/L)	1.037 (0.948-1.134)	0.428	Removed	Removed

Ref: Reference category; Variables wherein *p* of crude OR is in bold font were included in the multiple logistic regression analysis

Supplementary Table B. WHO Classification of COVID-19 based on severity

Mild Illness	Symptomatic patients presenting with fever, cough, fatigue, anorexia, myalgias; other non-specific symptoms such as sore throat, nasal congestion, headache, diarrhea, nausea and vomiting; loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms with NO signs of pneumonia or hypoxia
Moderate Illness	Patients with clinical signs of non-severe pneumonia (e.g., fever, cough, dyspnea, respiratory rate (RR) = 21-30 breaths/minute, peripheral capillary oxygen saturation (SpO_2) >90% on room air)
Severe Illness	With clinical signs of severe pneumonia or severe acute respiratory infection as follows: fever, cough, dyspnea, RR>30 breaths/ minute, severe respiratory distress or SpO_2 <90% on room air
Critical Illness	Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction