Abstract

Background: Vitamin D3 (1,25(OH)2cholecalciferol) as a treatment for COVID-19 patients is being disputed, and a clear clinical benefit is not being confirmed.

Methods: A retrospective evaluation for COVID-19 patients who were treated with various cumulative doses of vitamin D. Data was extracted from the COVID-19 database, it included patients admitted to three hospitals in Amman, Jordan. Characteristics of patients were tabulated and compared for all-cohort, and propensity score index (PSI) adjustment. The comparison was based on two vitamin D strata (≤ 149,000 i.u. and > 150,000 i.u.). Logistic regression analysis was utilized to predict recovery, the need for oxygen, and all-cause mortality for all-cohort, IPSW, and IPTW patients, based on vitamin D cumulative doses during their hospital stay.

Results: 1131 all-cohort and 768 PSI-adjusted patients were recruited. Except for antibiotics and antivirals, all other characteristics were balanced (P = NS). There were 1017 patients on vitamin D, 847 received cumulative ≤ 149,000 i.u., and 170 patients received cumulative dose
Introduction
COVID-19 hit the world populations causing morbidities and mortalities, cure and/or mitigation are being sought. Several interventions are adopted in the treatment of COVID-19 without strong evidence, like an effective antiviral therapy [1-3], zinc and ascorbic acid [4], and convalescent plasma [5, 6]. Vitamin D3 (1,25(OH)2 cholecalciferol) is being used in COVID-19 patients for its known immunomodulating effects, and its role in signaling during adaptive and innate immune responses in viral, and bacterial infections [7]. Previous studies associated the initial high serum levels, and vitamin D supplementations with reduced incidence of seasonal influenza or improvement in the duration and severity [8-10].

A large population-based observational study included 39,190 COVID-19 patients with low serum vitamin D levels (<20 ng/ml) had had higher positivity for SARS-CoV-2 (12.5%) compared with 27,870 patients with higher levels where they had lower positivity (8.1%), even 12,321 patients with values ≥55 ng/mL had positivity rates of 5.9%, in a multivariate analysis, the association rates between positivity rates and higher circulation Vitamin D was demonstrated (AOR 0.984 per ng/ml increment, P < 0.001) [11]. However, the use of vitamin D in COVID-19 patients have been linked to uncertainty, evidence supporting its use appeared as it was derived from a small RCT study detecting a reduction in COVID-19 severity in ICU patients, similar evidence came from other observational studies [12-14].

In the current study, we aim to illustrate the treatment effects of using vitamin D in COVID-19 patients. Our patients were prescribed different and varying doses during their hospital stay, to avoid confusion about regimens the total cumulative doses for vitamin D were evaluated to predict causal treatment effects.

Materials and Methods

Study Settings
Data for COVID-19 patients was collected from three participating hospitals (The Specialty, Jordan, and Al Khalidi) with a total bed capacity of around 700, special units for the management of patients with COVID-19 were allocated with an approximate capacity of 155-floor beds and 47 ICU beds. The study was a retrospective cross-sectional over 22 weeks (28 November 2020 to 6 May 2021), data was uploaded into a cloud excel sheet (Microsoft Corporation). Records were included as patients presented for admissions in the participating hospit-
The study was approved by each of the internal review boards of the three hospitals, no consent was needed.

Treatment protocols
There is a current updated COVID-19 management protocol published by the Jordan Ministry of Health (MOH). In the three hospitals, the treating physicians partially relied on the MOH protocol and literature updates, the treatment protocol for vitamin D was heterogeneous (supplementary material); different doses, and may vary over the treatment period in the same patient. Vitamin D tablets (used as a three-day 50,000 I.U. regimen before March 15, then a seven-day 50,000 I.U., also a 2000 I.U. and 5000 I.U., daily were prescribed. Other agents administered; steroids (dexamethasone or solumedrol), anticoagulants (Enoxaparin sodium, Apixaban, Rivaroxaban, and Fondaparinux), antivirals (Favipiravir, Remdesivir), acetylsalicylic acid (ASA), colchicine, Zn tablets, and Vitamin C (supplementary material).

Classification of radiological findings
Chest radiography scoring system: the degree of lungs involvement was based on radiologists’ classification for the degree of lungs involvement. Normal chest x-ray and/or normal CT chest with no infiltrate were considered as no involvement (score 1), a lobar infiltrate with 25% involvement (score 2), scattered ground glass appearance involving lungs with >25 - 50% involvement (score 3), diffuse patchy infiltrate >50 - 75% involvement was considered as (score 4), and multilobe infiltrate was considered as >75% involvement (score 5) [15].

Statistical analysis
Characteristics for the all-cohort and PS-adjusted patients were described, propensity-score match tolerance (caliper) was 0.1, without replacement, normal distribution for predictors was tested by skewness, histogram, P-P blot, and Q-Q blot, all fitted a normal curves distribution, skewness for all were < 1.0 and > -1.0, multicollinearity was evaluated by linear regression (tolerance was above 0.251, mostly > 0.8 and VIF was mostly less than 1.3 except two with 3.554 and 3.992), continuous predictors were evaluated by Levine’s test and were homoscedastic. Predicted probability was derived from continuous and binary predictors thru binary logistic regression analysis, some continuous predictors were Log10-transformed to normalize the distribution before they were incorporated in the model (supplementary material). Predictors entered were: Age, gender, the sum of recorded symptoms, comorbidities, Log_BMI, Tobacco, Log_ferritin level, Log_D-Dimer, Log_LDH, imaging scores, steroids, antivirals, antibacterials, Antifungal, IL6-inhibitors, documented temperature, blood oxygen saturation, oxygen delivery method, and peripheral white blood cells [16, 17]. Vitamin D treatment was analyzed by Chi-square test ($\chi^2$) astwo categories (<149,000, > 150,000) with post hoc analysis by Bonferroni adjusted p-value to assure balanced variables. IPSW patients were estimated for the outcome effects by Logistic regression analysis, it was tabulated for the all-cohort, PSM, and SIPTW patients. SPSS version 25 with Python Essentials and Fuzzy extension command blog-ins was used in the analysis, significance was considered for values < 0.05.

Results
The characteristics of the 1220 patients were reviewed, eighty-nine cases had missing data and were excluded. Analysis was for 1131 as the all-cohort, and 768 PSM and 768 PS-adjusted patients (Table 1). The differences in characteristics between two vitamin D dose categories among the all-cohort were balanced with the PSI (Table 1), few characteristics had significant (P < 0.05) but borderline imbalance with PS-adjustment. Age was balanced in the initial cohort except for the age group (Lowest thru 65 years), more patient proportions were in the higher vitamin D cumulative dose group (≤ 149,000 i.u.
Table 1. The characteristics of COVID-19 patients according to Vitamin D treatment allocations.

| Characteristic               | All cohort, Vitamin D | Propensity score-adjusted patients |
|------------------------------|-----------------------|------------------------------------|
|                              | N = 1017              | N = 768                            |
|                              | ≤ 149,000 i.u.        | ≥ 150,000 i.u.                     |
|                              | n = 847               | n = 170                            |
|                              | P *                   | ≤ 149,000 i.u.        | ≥ 150,000 i.u. |
|                              |                       | n = 639               | n = 129        |
| Age (years)                  |                       |                                   |                |
| Lowest thru 65               | 481 (56.9)            | 372                                |
| 66 thru 75                   | 194 (24.0)            | 139                                |
| 76 thru highest              | 171 (24.0)            | 128                                |
| Gender                       |                       |                                   |                |
| Male                         | 544 (65.9)            | 413                                |
| Female                       | 303 (65.9)            | 226                                |
| Antivirals                   |                       |                                   |                |
| Male                         | 537 (67.3)            | 443                                |
| Female                       | 303 (67.3)            | 226                                |
| Antibiotics                  |                       |                                   |                |
| Male                         | 656 (77.4)            | 504                                |
| Female                       | 81 (47.6)             | 62                                 |
| Antifungal                   |                       |                                   |                |
| Comorbidities                |                       |                                   |                |
| Chronic lung disease         | 11 (1.9)              | 11                                 |
| Heart disease                | 10 (2.5)              | 9                                  |
| Hypertension                 | 70 (12.0)             | 51                                 |
| Malignancy                   | 7 (2.0)               | 4                                  |
| Tobacco use                  | 99 (12.0)             | 80                                 |
| BMI                          |                       |                                   |                |
| Comorbidities                |                       |                                   |                |
| Chronic lung disease         | 11 (1.9)              | 11                                 |
| Heart disease                | 10 (2.0)              | 9                                  |
| Hypertension                 | 70 (12.0)             | 51                                 |
| Malignancy                   | 7 (1.2)               | 4                                  |
| Tobacco use                  | 99 (12.0)             | 80                                 |
| Blood oxygen saturation (%)  |                       |                                   |                |
| >94                          | 174 (34.0)            | 117                                |
| 90 – 94                      | 286 (50.0)            | 216                                |
| 85 – 89                      | 184 (35.0)            | 149                                |
| 80 – 84                      | 102 (19.0)            | 85                                 |
| <80                          | 88 (17.0)             | 72                                 |
| Oxygen delivery method       |                       |                                   |                |
| RA                           | 121 (15.0)            | 88                                 |
| Simple mask                  | 108 (26.0)            | 72                                 |
| High flow                    | 25 (11.0)             | 20                                 |
| Noninvasive ventilation      | 34 (4.0)              | 29                                 |
| Combined                     | 36 (7.0)              | 30                                 |
| IMV%                         | 31 (2.0)              | 24                                 |
| Non-rebreather mask          | 168 (30.0)            | 142                                |
| Nasal Prongs                 | 309 (53.0)            | 245 (36.6)                         |

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= 56.9% versus ≥ 150,000 i.u. = 65.9%, p < 0.05), however, all other categories were balanced in the PS adjustment. Gender remained well-balanced in the initial cohort (P = 0.542) and the PS-adjusted patients (P = 0.817). Antivirals and antibiotics were imbalanced in both all-cohort and the PS-adjusted patients (P < 0.05). Documented fever, comorbidities, BMI, tobacco use, and blood oxygen saturation were well balanced for both cohorts (P = NS).

Oxygen delivery method was balanced for the all-cohort patients and PS-adjustment (P = NS) except for the nasal prongs, it remained imbalanced (P < 0.05) where more patients were in the vitamin D category > 150,000 i.u. Admission radiological imaging showed well balance in the all-cohort and PS-adjusted patients except it were imbalanced for the >75% involvement. PCT levels were well balanced in both cohorts (P = NS). Serum ferritin was imbalanced for the subcategory (<260 ng/ml) in the PS-adjusted patient, both cohorts were well balanced (P = NS).

There were 1017 patients on vitamin D, 847 received cumulative ≤ 149,000 i.u., and 170 patients received cumulative dose ≥ 150,000 i.u. (Dose range 1000 – 385000), and the majority (823) was in ≤ 50,000 cumulative i.u.

**Outcomes analyses**

In the all-cohort analysis, recovery was in 401 patients, vitamin D supplement increasing cumulative dose did not significantly contribute to recovery (B = 0.000, S.E = 0.000, Wald = 4.076, P = 0.043, OR = 1.000, 95% C.I. 1.000 to 1.000), also, no significance was noted for IPSM (B = 0.000, S.E = 0.000, Wald = 187.905, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000), and IPTW (B = 0.000, S.E = 0.000, Wald = 278.483, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000). There were 455 patients...
discharged home on oxygen, no observable effect of vitamin D to avoid patients from using home oxygen (B = 0.000, S.E = 0.000, Wald = 16.421, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000), similarly no significance was for IPSM analysis (B = 0.000, S.E = 0.000, Wald = 164.703, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000), and IPTW (B = 0.000, S.E = 0.000, Wald = 207.543, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000).

Overall, all-cause mortality was 13.79% (156 patients), and for those who stayed in the ICU the mortality was 55.0% (110 patients). There was no significant effect for the cumulative doses of vitamin D on all-cause mortality (B = 0.000, S.E = 0.000, Wald = 4.847, P = 0.028, OR = 1.000, 95% C.I. 1.000 to 1.000), similarly no significance was noted for IPSM analysis (B = 0.000, S.E = 0.000, Wald = 8.386, P = 0.004, OR = 1.000, 95% C.I. 1.000 to 1.000), and IPTW (B = 0.000, S.E = 0.000, Wald = 20.509, P = 0.000, OR = 1.000, 95% C.I. 1.000 to 1.000) (Table 2).

Discussion
With the advent of the COVID-19 pandemic and its high morbidity and mortality including more clot-

Table 2. The outcome of using Vitamin D in the treatment of the COVID-19 patients analyzed as all-cohort, propensity score adjustment, and inverted probability of treatment weight (IPTW) by logistic regression analysis.

| Characteristic       | B       | S.E.     | Wald    | P       | OR (ExpB) | 95% C.I. |
|----------------------|---------|----------|---------|---------|-----------|----------|
| Recovery*            |         |          |         |         |           |          |
| All-cohort           | 0.000   | 0.000    | 4.076   | 0.043   | 1.000     | 1 to 1   |
| IPSW                 | 0.000   | 0.000    | 187.905 | 0.000   | 1.000     | 1 to 1   |
| IPTW                 | 0.000   | 0.000    | 278.483 | 0.000   | 1.000     | 1 to 1   |
| On home oxygen       |         |          |         |         |           |          |
| All-cohort           | 0.000   | 0.000    | 16.421  | 0.000   | 1.000     | 1 to 1   |
| IPSW                 | 0.000   | 0.000    | 164.703 | 0.000   | 1.000     | 1 to 1   |
| IPTW                 | 0.000   | 0.000    | 207.543 | 0.000   | 1.000     | 1 to 1   |
| All-cause Mortality  |         |          |         |         |           |          |
| All-cohort           | 0.000   | 0.000    | 4.874   | 0.028   | 1.000     | 1 to 1   |
| IPSW                 | 0.000   | 0.000    | 8.386   | 0.004   | 1.000     | 1 to 1   |
| IPTW                 | 0.000   | 0.000    | 20.509  | 0.000   | 1.000     | 1 to 1   |

Percent in (brackets) are proportions of sub-variables when statistical significance was demonstrated. *2-sided Significance (P-value) was tested by χ2, and Bonferroni method. % Invasive mechanical ventilation. NS: not significant.
COVID-19 patients (OR = 1, 95% CI. 1 to 1). Also, it did not improve the rates of the need for home oxygen therapy for discharged patients (OR = 1, 95% CI. 1 to 1). The all-cause mortality in our patients was 13.79% and the ICU mortality was 55%. However, patients analyzed as all-cohorts, IPSW and IPTW did not show any improvement with the increasing vitamin D cumulative dose (OR = 1, 95% CI. 1 to 1).

Conclusion

In our patients’ cohort, we could not demonstrate a beneficial effect for vitamin D as a therapy in COVID-19 patients for the measured outcomes; recovery by hospital discharge, the need for home oxygen therapy, and all-cause mortality. Those results confirm previous literature that demonstrated no clear clinical effects for using vitamin D as a therapy.

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