Research Article

Reyhan Öztürk*, Niyazi Samet Yılmaz and Mustafa Ulukanlıgil

The relationship between serum vitamin D and antibody response following two doses of inactivated COVID-19 vaccine

https://doi.org/10.1515/tjb-2022-0123
Received May 30, 2022; accepted August 1, 2022; published online August 18, 2022

Abstract

Objectives: Low vitamin D levels are associated with the severity and mortality of COVID-19 infection. Nevertheless, the relationship between the 25-hydroxyvitamin D [25(OH)D] levels and the antibody response following COVID-19 vaccination is not fully elucidated. Herein, we explored the relationship between SARS-CoV-2 IgG (sCOVG) and 25(OH)D.

Methods: In this prospective observational case-control study, we used an automated chemiluminescent immunoassay method to measure sCOVG and 25(OH)D levels in 96 patients 28 days following the second dose of inactivated vaccine. We considered the positivity for sCOVG at three different index values: 1, 2.42, and 7. We classified 25(OH)D levels between 0 and 20 ng/mL as vitamin D deficiency, 21–29 ng/mL as insufficiency and 30 ng/mL as sufficiency.

Results: Median sCOVG index was 6.02 (interquartile ranges 3.41–11.63) and median 25(OH)D level was 11.5 ng/mL (interquartile ranges 10–17). We could not find a significant correlation between 25(OH)D and sCOVG levels (Spearman’s rho, r=0.175, p=0.12). When considering the variables categorically, we did also not conclude significant relationships between adequate or inadequate antibody responses in patients with deficient, insufficiency and sufficient 25(OH)D by three sCOVG cut-off index values (1, 2.42, and 7) (Chi-square test, p=0.8, 0.29, and 0.08, respectively).

Conclusions: The relevant literature is limited on the association between the antibody response to COVID-19 vaccines and vitamin D levels. Although the previous research suggested conflicting findings of the response to mRNA vaccines, we could not conclude a significant relationship between sCOVG and 25(OH)D levels 28 days after two doses of inactivated COVID-19 vaccine.

Keywords: 25-hydroxyvitamin D; COVID-19; SARS-CoV-2; SARS-CoV-2 IgG; vaccination.

Introduction

Micronutrients regulate the innate and adaptive immune response to viral diseases such as COVID-19 caused by the novel type of coronavirus, SARS-CoV-2 [1]. In this regard, vitamin D is commonly associated with reducing the cytokine storm occurring during viral infections and enhancing the antiviral innate or adaptive immune response [2]. Such an immunomodulatory role attributed to vitamin D stems from its receptors on many immune cells (e.g., macrophages, monocytes, dendritic cells, T cells, and B cells) and the ability of these cells to convert it from its storage form, 25-hydroxyvitamin D [25(OH)D], to its active form, 1,25-(OH)2-hydroxyvitamin D [3]. As a consequence of the activation of vitamin D by immune cells, active vitamin D protects mucosal integrity in inflamed tissues and boosts the synthesis of antimicrobial peptides, cathelicidin, and defensins [4–6]. Additionally, the previous research showed that vitamin D suppresses the proinflammatory cytokine profile synthesized from Th1 and Th17 cells and regulates the inflammatory cytokine profile by stimulating Th2 and Treg cells [4–7]. The SARS-CoV-2 virus infects type 2 pneumocytes through angiotensin-converting enzyme-2 (ACE2) receptors. The occurrence of infection in other organs is also associated with ACE2 receptor expression [8]. Vitamin D also acts as a negative regulator in the renin-angiotensin-aldosterone

*Corresponding author: Reyhan Öztürk, MD, Infectious Diseases and Clinical Microbiology, Polatlı Duatepe State Hospital, Polatlı Duatepe Devlet Hastanesi, İl Hastalıkları Kliniği, Karapınar, Eskişehir yolu üzerinde Abdülaziz Cad. No:2, 06900 Polatlı/Ankara, Ankara, Turkey, Phone: +90 544 426 32 56, E-mail: dreyhan@hotmail.com. https://orcid.org/0000-0002-0969-3961
Niyazi Samet Yılmaz, Medical Biochemistry, Polatlı Duatepe State Hospital, Ankara, Turkey. https://orcid.org/0000-0002-4336-2517
Mustafa Ulukanlıgil, Medical Microbiology, Polatlı Duatepe State Hospital, Ankara, Turkey. https://orcid.org/0000-0002-0316-193X
axis, and—thanks to this effect—it appears to be a protective factor in COVID-19 infection [9].

Since the beginning of the COVID-19 pandemic, a substantial number of scientific studies and meta-analyses have investigated the link between vitamin D levels and the severity of COVID-19 infection, lung involvement rates, and the need for intensive care, intubation rates, and mortality rates. Despite varying effect sizes among the findings, meta-analyses uncovered that 25(OH)D levels remain lower in COVID-19 patients than in control subjects, in patients with severe COVID-19 infection compared to those with mild COVID-19 infection, in patients needing intensive care compared to those not, and in cases with mortality compared to survivors [10–13]. Although the literature hosts extensive findings on the relationship between COVID-19 infection and vitamin D, the association between antibody response and vitamin D levels after COVID-19 vaccination is not yet clear. Thus, this prospective observational case-control study explored the relationship between SARS-CoV-2 IgG (sCOVG) and 25(OH)D levels following two doses of inactivated COVID-19 vaccine.

Materials and methods

Research design and sample

We conducted the study fully complying with the principles of the Declaration of Helsinki. The Ministry of Health, Scientific Research, and Evaluation Commission for COVID-19 and the Ethics Committee of Etlik Zubeyde Hanım Gynecology Training and Research Hospital granted ethical approval to our study (2022/10 dated 01.21.2022). Moreover, we sought relevant permissions for the study from the administration of Ankara Polatlı Duatpe State Hospital and obtained written informed consent from all participants. Overall, we recruited 124 consecutive adults, aged between 18 and 65, admitted to the Infectious Diseases outpatient clinic for sCOVG measurement 28 days after the second dose of the COVID-19 vaccine (CoronaVac, Sinovac, Beijing, China) without a history of COVID-19 infection and no evidence of active infection. Patients with known systemic disorders (n=21), patients with disorders affecting calcium and vitamin D metabolism (n=1, hypoparathyroidism), pregnancy or lactation, patients with a history of malabsorption (n=2), patients who had a history of antiepileptic, glucocorticoid (n=1) or 25(OH)D supplement use (n=3) were excluded from the study, and we analyzed the data of 96 patients. We recorded demographics, including age, gender, height, weight, body mass index (BMI), comorbidities (including systemic disorders, metabolic bone disorders, and disorders affecting calcium and 25(OH)D metabolism), pregnancy and lactation, medication use (including anticonvulsants, glucocorticoids and 25(OH)D replacement), COVID-19 infection, and vaccination history. We collected venous blood samples from the patients 28 days after the second dose of the vaccine to measure sCOVG and 25(OH)D levels.

Measurement of SARS-CoV-2 IgG (sCOVG) levels

We measured sCOVG levels using the chemiluminescent immunoassay method with the Siemens ADVIA Centaur SARS-CoV-2 IgG kit (Cat. No.: 11,207,376, Rev. 01, 2020–09) in the ADVIA Centaur XPT system (Siemens, Munich, Germany). This assay helps measure IgG levels developed against the spike receptor-binding domain (S1-RBD) antigen. Previously, it was found that sCOVG levels are strongly correlated with viral neutralizing antibody titers [14]. The measuring range of the test is between an index value of 0.5–150; however, for measurements outside the range, the upper limit of the measurement can be extended up to an index value of 750, thanks to automatic dilution in the XPT system. While within-study and within-laboratory variances became ≤12% and ≤15%, respectively, for an index value between 0.8 and 2, they were ≤10% and ≤12%, respectively, for an index value >2.0. Although the company specifies the positivity cut-off value as an index value of 1, the previous research also used 2.42 and 7 as cut-off values. In this study, we considered positivity by three different cut-off values [14–16].

Measurement of 25-hydroxyvitamin D [25(OH)D]

Using the chemiluminescent immunoassay method, we measured 25(OH)D levels with the Siemens ADVIA Centaur automated total 25(OH)D Vitamin D kit (Cat. No.: 10,699,201, Rev. G, 2020–09) in the ADVIA Centaur XPT system (Siemens, Munich, Germany). The measurement range of the test is 4.0–150 ng/mL. The manufacturer reports within-study and total variances to be ≤8% and ≤12%, respectively. We classified 25(OH)D levels between 0 and 20 ng/mL as vitamin D deficiency and between 21 and 29 ng/mL as vitamin D insufficiency while considering values of 30 ng/mL and above sufficient [17].

Statistical analysis

We considered skewness-kurtosis values, extreme values, normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests), and histogram graphs to check the normality of distribution. The data were presented as medians and interquartile ranges (IQR) or as numbers and percentages. While we explored the relationships between the categorical variables through the Chi-square test, we performed Mann-Whitney U and Kruskal-Wallis H tests to compare the data between the groups. Moreover, we calculated Spearman’s correlation coefficients and binary logistic regression analyses to examine the relationships between the variables. All analyses were performed on Statistic Program for Social Sciences version 23 (SPSS 23, IBM Corp., Armonk, NY, USA), and a p-value<0.05 was considered statistically significant.

Results

The present study included 25 (26%) male and 71 (74%) female subjects with a median age of 40 years (IQR=34–45 years) and a median BMI of 24.9 kg/m² (IQR=22.8–26.5 kg/m²). Median sCOVG index was 6.02 (IQR=3.41–11.63); 94 (97.9%) patients (by index value of 1), 79 (82.3%) patients (by index value of 2.42), and 38 (39.6%) patients
(by index value of 7) had adequate antibody levels after two doses of vaccination. Median 25(OH)D level was 11.5 ng/mL (IQR=10–17 ng/mL). 79 (82.3%) patients were 25(OH)D deficient, 9 (9.4%) had vitamin D insufficiency, and 8 (8.3%) participants had sufficient vitamin D levels (Table 1). We have created categorical subgroups for age (40 years and below, over 40 years of age), BMI (below and above 25 kg/m²), and 25(OH)D (below and above 20 ng/mL) for further analysis.

Gender and BMI did not affect sCOVG index values; however, the patients aged 40 years and below (n=49) had significantly higher sCOVG index values than those over 40 (n=47). Accordingly, the median sCOVG index values were determined to be 7.94 for the patients aged 40 years and below (IQR=4.71–14.35) and 4.38 for those aged above 40 years (IQR=2.4–7.39) (p=0.002, Mann-Whitney U test). Similar results were observed when sCOVG levels and age were categorically compared with chi-square tests; patients above 40 years had lower positivity for sCOVG cut-offs of 2.42 and 7 (p=0.03 and 0.01, respectively, x² tests) (Table 2). 25(OH)D levels did not differ significantly by gender, age, or BMI.

A logistics regression analysis revealed that patients aged over 40 years had a lower OR for sCOVG indexes higher than 7 (OR 0.67, 95% confidence interval 0.15–0.85). Gender, age, BMI, and 25(OH)D levels did not show significant relationships between adequate or inadequate antibody responses in patients with sCOVG index values of 2.42 and 7 (Table 2). Median sCOVG levels did not differ significantly in 25(OH)D deficient, insufficient, and sufficient patients (5.57, 6.96, and 15.32, respectively, Kruskal-Wallis H test, p=0.102) (Figure 1); and we could not conclude to a significant correlation between 25(OH)D and sCOVG levels (r=0.175, p=0.12).

Table 1: Population characteristics (n=96).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>25 (26%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>71 (74%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>≤40</td>
<td>40 (34–45)</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>47 (48.9%)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65 (62–75)</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>162 (158–172)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>BMI ≤25</td>
<td>48 (50%)</td>
</tr>
<tr>
<td></td>
<td>BMI ≥25</td>
<td>48 (50%)</td>
</tr>
<tr>
<td>sCOVG (index)</td>
<td>&gt;1 index</td>
<td>94 (97.9%)</td>
</tr>
<tr>
<td></td>
<td>&gt;2.42 index</td>
<td>79 (82.3%)</td>
</tr>
<tr>
<td></td>
<td>&gt;7 index</td>
<td>38 (39.6%)</td>
</tr>
<tr>
<td>25(OH)D, ng/mL</td>
<td>≤20 ng/mL</td>
<td>11.5 (10–17)</td>
</tr>
<tr>
<td></td>
<td>20–29 ng/mL</td>
<td>79 (82.3%)</td>
</tr>
<tr>
<td></td>
<td>≥30 ng/mL</td>
<td>8 (8.3%)</td>
</tr>
</tbody>
</table>

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; sCOVG, SARS-CoV-2 IgG; aData are presented as median (interquartile ranges); bData are presented as n (%).

Discussion

The immunomodulatory aspect of vitamin D in viral infections is well established, and the literature hosts a plethora of meta-analyses on the association between the severity of COVID-19 infection and vitamin D levels and supplementation. It was previously suggested that sufficient vitamin D levels or supplementation would play a role in the COVID-19 vaccine response; nevertheless, the research seems to have missed the relationship between 25(OH)D levels and antibody response to inactivated COVID-19 vaccine [18, 19]. The present prospective observational case-control study concluded no relationship between the participants’ 25(OH)D and sCOVG levels evaluated simultaneously 28 days after two doses of inactivated COVID-19 vaccine. When comparing those developing and not developing adequate antibody response by three different sCOVG cut-off values, we found the distribution of the patients with deficient, insufficient, and sufficient 25(OH)D levels to be similar. In addition, median sCOVG levels did not differ significantly in 25(OH)D deficient, insufficient, and sufficient patients. Moreover, both correlation and regression analyses failed to show an effect of 25(OH)D levels on sCOVG levels. Accordingly, our findings suggest that there is likely no link between adequate antibody response to inactivated COVID-19 vaccine and vitamin D levels.

Whereas some studies proposed that vitamin D levels positively impact viral vaccine response, others suggested the opposite. A randomized placebo-controlled study exploring the association between hepatitis B vaccine response and vitamin D levels showed that low vitamin D levels were associated with a poorer vaccine response and that supplementation starting three days after vaccination did not affect the vaccine response [20]. Similarly, in a study on chronic renal failure patients, a population commonly suffering from vitamin D deficiency, the hepatitis B seroconversion rate in patients with 25(OH)D levels ≥10 ng/mL was found to be greater than in those with vitamin D levels <10 ng/mL. It was also reported that vitamin D deficiency in patients with chronic kidney disease was associated with weak antibody formation after
Table 2: Summary of chi-square tests and binary logistic regression analysis predicting the sCOVG levels in the study group.

<table>
<thead>
<tr>
<th></th>
<th>sCOVG&lt;2.42 index</th>
<th>sCOVG≥2.42 index</th>
<th>p-Valuea</th>
<th>OR (95% CI)</th>
<th>p-Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (29.4%)</td>
<td>20 (25.3%)</td>
<td>0.46</td>
<td>0.81 (0.25–2.59)</td>
<td>0.72</td>
</tr>
<tr>
<td>Female</td>
<td>12 (70.6%)</td>
<td>59 (74.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40</td>
<td>5 (29.4%)</td>
<td>44 (55.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>12 (70.6%)</td>
<td>35 (44.3%)</td>
<td>0.06</td>
<td>0.33 (0.10–1.03)</td>
<td>0.056</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>6 (35.3%)</td>
<td>42 (53.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥25</td>
<td>11 (64.7%)</td>
<td>37 (46.8%)</td>
<td>0.28</td>
<td>0.48 (0.16–1.42)</td>
<td>0.18</td>
</tr>
<tr>
<td>25(OH)D, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 ng/mL</td>
<td>16 (94.1%)</td>
<td>16 (20.3%)</td>
<td>0.15</td>
<td>4.06 (0.5–32.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>≥20 ng/mL</td>
<td>1 (5.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sCOVG, SARS-CoV-2 IgG; x², chi-square test; OR, odds ratio; CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; a p-values of chi-square tests; b p-values of binary logistic regression analysis. Data are presented as n (%).

The hepatitis B vaccine [21]. Besides, another study showed that 1,25-(OH)2 Hydroxyvitamin D administered with the adjuvant to the inactivated poliovirus vaccine increased the systemic and mucosal immunity in mice and recommended a possible use of vitamin D as an adjuvant [22]. Contrarily, a randomized controlled study evaluating the effect of vitamin D supplementation among older adults receiving influenza vaccination concluded that increased plasma TGF-β levels and reduced Th1/Th2 ratio compared to controls 28 days after vaccination but no difference between antibody levels [23]. A meta-analysis on the relationship between influenza vaccine response and vitamin D showed that although vitamin D levels were not associated with post-vaccine seroconversion and protective antibody titer, there might be differences by influenza type and that vitamin D deficiency created a protective

Figure 1: Comparison of SARS-CoV-2 IgG (sCOVG) index levels of patients with 25-hydroxyvitamin D [25(OH)D] deficiency, insufficiency, and sufficiency. Data are presented as median and interquartile ranges.
antibody titer in fewer patients in influenza A H3N2 (A/H3N2) and influenza B [24]. Contrary to the findings in the literature, higher antibody titers were detected in patients with low vitamin D levels in HPV vaccination. The authors compared this finding to low vitamin D levels often seen in patients with exaggerated inflammatory responses in conditions such as multiple sclerosis and inflammatory bowel disease [25].

The literature still offers limited findings on the association between the antibody response to COVID-19 vaccines and vitamin D levels. Previously, age and vitamin D levels were shown to be critical factors in the antibody response to the Pfizer/BioNTech COVID-19 mRNA vaccine following the first dose [26]. In support of the present study, a preprint of a population-based cohort study in the United Kingdom revealed that one of the factors affecting the antibody response to 2 doses of mRNA vaccine was routine vitamin D supplementation reported by participants [27]. However, contrary to our findings, a population-based cohort study on healthy German healthcare workers concluded no correlation between the antibody response following the first or second dose of mRNA vaccine and the maximum sCOVG levels or decreased sCOVG levels and 25(OH)D levels [17]. As a contribution to the available findings in the literature, we also reached no correlation between sCOVG levels acquired 28 days after two doses of inactivated COVID-19 vaccine and simultaneously acquired 25(OH)D levels. These conflicting results may be due to the types of vaccines used, sample characteristics, and methodological differences. Yet, it is evident that prospective randomized controlled studies are needed on different age and patient groups to uncover the relationship between 25(OH)D levels and vaccine response.

Our study identified age as a factor that affects sCOVG levels 28 days after two doses of inactivated COVID-19 vaccine. Both median sCOVG levels were found to be lower in patients aged above 40 years, and regression analyses identified that for both sCOVG ≥ 2.42 and sCOVG ≥ 7 cut-offs, patients above 40 years had a lower frequency of adequate antibody response. These results were consistent with the previous overwhelming evidence regarding antibody response to different COVID-19 vaccines, as previous studies also identified age as a detrimental factor to the development of adequate antibody response and antibody persistence [28–30].

**Limitations**

Our study is not free of a few limitations. Above all, the observational nature of the study is relatively constraining. A consecutive sampling method was employed, and post-hoc study power was 47.5%, calculated using endpoint mean values. Furthermore, previous studies were conducted on antibody response to the COVID-19 mRNA vaccines, whereas the main focus of the current study was the relationship between sCOVG and 25(OH)D levels following two doses of inactivated COVID-19 vaccine. Moreover, we used only the sCOVG index, a humoral marker, to measure adequate vaccine response and did not consider cellular immunity. In this single-center study, we could also not reexamine sCOVG and 25(OH)D levels in different time periods due to the small number of patients and financial constraints, which made us unable to perform further analysis. Thus, our findings are better to be addressed considering these limitations.

**Conclusions**

Overall, we could not conclude a significant relationship between 25(OH)D and sCOVG levels measured simultaneously 28 days after two doses of inactivated COVID-19 vaccine. Both categorical comparisons between adequate or inadequate antibody response by different sCOVG cut-off index values and deficient, insufficient, and sufficient vitamin D levels with regression analyses; and median sCOVG level comparisons with non-parametric tests and correlation analyses failed to show an effect of 25(OH)D levels on sCOVG levels. Consistent with the previous evidence, we have identified that older patients had lower sCOVG levels, hence a reduced adequate antibody response to inactivated COVID-19 vaccine. Further prospective randomized controlled studies are needed to explore the relationship between 25(OH)D levels and vaccine response in different age and patient groups.

**Research funding:** The funding of this study was covered by the researchers.

**Author contributions:** Research idea/concept: RO, NSY, MU; Design: RO, NSY, MU; Supervision/consultancy: RO; Data collection and/or analysis: NSY, MU, RO; Analysis/interpretation: RO, NSY, MU; Literature review: RO, NSY, MU; Manuscript writing: RO; Critical review: RO, NSY, MU; Resources and funding: RO, NSY, MU; Materials: RO, NSY, MU. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Competing interests:** The authors state no conflict of interest.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Ethical approval:** We carried out the study fully complying with the principles of the Declaration of Helsinki. The Ministry
of Health, Scientific Research and Evaluation Commission for COVID-19 and the Ethics Committee of Edilık Zübeyde Hanım Gynecology Training and Research Hospital granted ethical approval to our study (2022/10 dated 01.21.2022). Moreover, we sought relevant permissions for the study from the administration of Ankara Polatlı Duatepe State Hospital.

References

immunogenicity, contributing factors of an immune response, and reactogenicities after a single dose of the ChAdOx1 (AZD1222) COVID-19 vaccine in the Thai population. Hum Vaccines Immunother 2022;18:2035573.
