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Abstract: Background/Objectives: Low blood vitamin D levels have been linked to COVID-19 severity; however, data on the effects of vitamin D on vaccine immunogenicity remain limited. This study evaluated the impact of baseline serum 1α, $25\text{-}(OH)_2$ vitamin D, a bioactive form with controlled blood concentrations, on vaccine response. **Methods**: From June to September 2021, we measured active vitamin D levels in 88 Japanese workers and students before vaccination with BNT162b2 or mRNA-1273. We assessed SARS-CoV-2 spike protein S1 IgG and IgM levels and antigen-specific INF-γ-releasing cells from pre-vaccination up to 8 months post-second dose. **Results**: Active vitamin D levels ranged from 33.7 to 99.8 pg/mL, 60.2% above the Japanese reference range (20–60 pg/mL). In a mixed model accounting for repeated measures and sub-cohort random effects and following fixed effects, vaccine type, weeks post-vaccination, age, sex, *ALDH2* gene polymorphism, height, smoking, ethanol intake, exercise, stress, steroid use, allergies, dyslipidemia, active vitamin D was negatively associated with anti-S1 IgG and IgM ($p < 0.001$) but positively with INF-γ-releasing cell counts ($p = 0.003$). **Conclusions**: As the robustness of cellular immunity is independent of viral mutations, $1,25\text{-}(OH)_2$ vitamin D levels above the reference range may enhance vaccine-induced COVID-19 protection in the long term.

Keywords: vitamin D; COVID-19; vaccine-Induced Immunity

1. Introduction

Vitamin D has a regulatory effect on the immune system [1,2], including innate and adaptive immunity in COVID-19 [3,4]. Low vitamin D levels are associated with the morbidity and severity of COVID-19 [5,6]; however, few studies have examined the association between blood vitamin D levels and COVID-19 vaccine immunogenicity. Saroha et al. (2024) analyzed the mRNA dynamics of peripheral blood mononuclear cells (PBMCs) and suggested that vitamin D supplementation

improves the efficacy of the viral vector vaccine ChAdOx1 nCoV-19 by enhancing T-cell memory responses [7].

In contrast, Lavell et al. reported no association between baseline precursor vitamin D concentrations and humoral or cellular immune responses to mRNA vaccines [8].

The precursor vitamin D, 25-hydroxyvitamin D, reflects the total amount of vitamin D [9], and its blood concentration shows high variability, most likely due to the amount of ultraviolet radiation exposure; the precursor vitamin D levels in summer are more than twice those in winter [10]. This high variability suggests the limitations of studies focusing on precursor vitamin D as the concentrations of precursor and active forms of vitamin D do not correlate [2]. The active form, 1, 25-$(OH)_2$ vitamin D, is produced via hydroxylation of 25(OH) vitamin D by 1α-hydroxylase (CYP27B1) in the kidneys [9], and parathyroid hormone keeps its blood levels constant at a range of 120–140 pmol/L (50–58 pg/mL) [10,11]. Active vitamin D binds to vitamin D receptors expressed in immune cells, migrates into the nucleus, and binds directly to genomic DNA, thereby regulating the expression of genes involved in the immune system [12], including monocytes [13], macrophages [14], dendritic cells [15], and T and B lymphocytes [16,17].

Studies on the relationship between active vitamin D and COVID-19 immunity are lacking. To address this gap, we examined the association between baseline blood active vitamin D levels and the humoral and cellular immunogenicity of COVID-19 mRNA vaccines in the general Japanese population.

2. Materials and Methods

This study was approved by the Ethics Committee for Clinical Research at the School of Medicine, Saga University, Saga, Japan (No. R2-44 and R3-9). All the participants provided written informed consent before undergoing any study procedure.

2.1. Study Design

The study group included 88 participants (43 males and 45 females) from hospitals and a university in Saga Prefecture who voluntarily received two doses of a COVID-19 mRNA vaccine. As shown in Figure 1, 62 participants in sub-cohorts 1 and 2 (20 healthcare workers: 10 males, 10 females; and 42 students: 22 males, 20 females) received two doses of BNT162b2 (Pfizer Inc., New York, NY, USA/BioNTech SE, Mainz, Germany) (30 μg). Sub-cohort 3, comprising 26 university employees and students (11 males, 15 females), received two doses of mRNA-1273 (Moderna Inc., Cambridge, MA, USA/Takeda Pharmaceutical Co., Ltd., Tokyo, Japan) (100 μg). The first dose was administered in April and May 2021 for sub-cohorts 1 and 2, and in August for sub-cohort 3, with the second dose given 21 days after the first for BNT162b2 and 28 days after for mRNA-1273. None of the participants had a history of COVID-19 at baseline or were pregnant. Data were excluded if SARS-CoV-2 infection was suspected during follow-up based on specific antibody and lymphocyte levels.
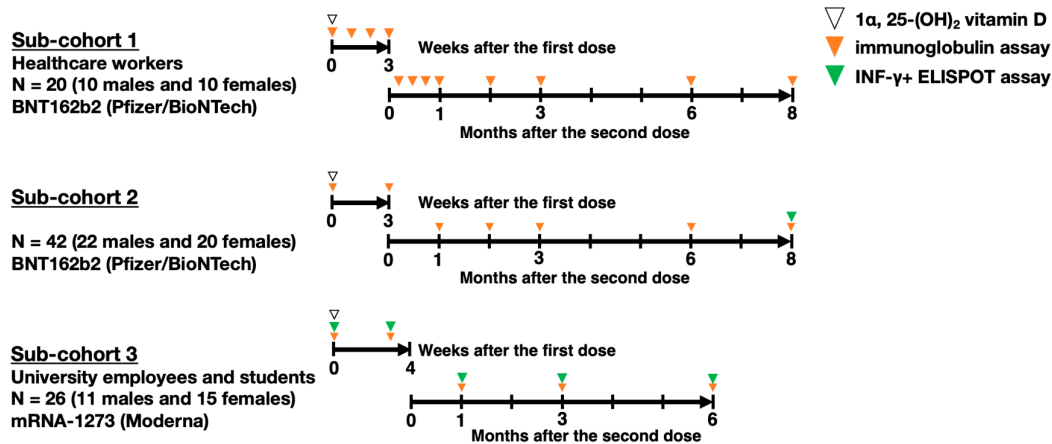
Figure 1. Study design and participants. The study group comprised 88 participants, 3 sub-cohorts from hospitals and a university in Saga Prefecture, vaccinated of their own will. The number of participants, vaccine type, and timing of blood collection are presented. The first dose was administered as follows: April, May, and August 2021 for sub-cohorts 1, 2, and 3, respectively; the second dose was administered 3 and 4 weeks after the first dose for BNT162b2 and mRNA-1273, respectively. None of the participants had history of COVID-19 at baseline. Blood collection for each analysis is indicated by the white, green, and orange arrowheads.

Blood samples were collected from the participants at multiple time points to monitor their immune responses to the COVID-19 mRNA vaccine. Initial samples were collected prior to the administration of the first vaccine dose to establish baseline measurements. Subsequent samples were collected, as shown in Figure 1, briefly, at weekly intervals for 3 weeks following the first dose in sub-cohort 1 or only 3 weeks later in sub-cohorts 2 and 3. After the second dose, blood samples were collected at weeks 1, 2, and 3 only in sub-cohort 1 and at 1 3-month intervals up to 8 months following the second dose in all sub-cohorts.

2.2. Serological Tests

Serum was extracted on the drawing day and stored at −80 ℃ until analysis. The active form of vitamin D, i.e., 1α, 25-(OH)$_2$ vitamin D, was quantified in a radioimmunoassay. A high-sensitivity chemiluminescent enzyme immunoassay platform (Sysmex Co., Kobe, Japan) was used to measure anti-SARS-CoV-2 antibodies, the S1 subunit of the anti-spike protein (S1 subunit) IgG, anti-S1 IgM, and anti-nucleocapsid protein (N) IgG to monitor SARS-CoV-2 viral infection [18]. The units for anti-S1 IgG, anti-IgM, and anti-N IgG were binding antibody units per milliliter (BAU/mL), Sysmex units per mL (SU/mL), and SU/mL, respectively. The BAU was calibrated using the WHO International Standards.

2.3. Enzyme-Linked Immunospot Assay

The numbers of antigen-specific INF-γ-releasing lymphocytes were determined as previously described [19]. Briefly, deep-frozen peripheral blood mononuclear cells donated from 26 participants in sub-cohort 3 were subjected to an Enzyme-Linked ImmunoSpot (ELISPOT) assay, followed by 19 additional ELISPOT assays, depending on the availability of research funding, for a subset of participants in sub-cohort 2, 8 months following the second dose. A human IFN-γ ELISPOT kit (Mabtech AB, Nacka Strand, Sweden) was used to evaluate cellular immunity against SARS-CoV-2 using lyophilized 315-peptide mixtures of SARS-CoV-2 spike glycoprotein, PepMixTM SARS-CoV-2 (JPT Peptide Technologies, Berlin, Germany), the original strain (PM-WCPV-S-2) as antigens. The peptide mixture was dissolved in DMSO at a concentration of 0.5 ug/peptide; DMSO was used as a negative control, while an NF-kB activator, phorbol myristate acetate, and ionomycin were positive

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controls. The assay was performed according to the manufacturer's instructions using a spot counter, ImmunoSpot S6 VERSA (Cellular Technology Limited, Shaker Heights, OH, USA).

2.4. Self-Administered Questionnaire

A self-administered questionnaire was used, as previously described [20]. Briefly, the participants were asked about cigarette smoking and habitual drinking. Alcohol consumption was calculated based on the amount consumed over the previous six months, adjusted for a body weight of 60 kg, and categorized into four groups: less than 1 g/day, 1 g/day or more, less than 20 g/day, and 20 g/day or more. Exercise habits were assessed by asking, "Do you usually exercise?" with response options of no habit, less than 1 day/week, 1 to 3 days/week, and 3 or more days/week. Perceived stress was evaluated using the question "Do you feel psychological stress?" on a 5-point scale: no (0), mostly no (1), unsure (2), quite often (3), and yes (4). The presence of steroid use, allergic diseases, and dyslipidemia over the past 3 years.

2.5. Genotyping of rs671

The East Asian-specific variant, a single nucleic polymorphism of *Aldehyde dehydrogenase 2* (*ALDH2*) rs671, was identified as previously described [20]. In brief, DNA was extracted from blood clots as follows: Approximately 0.1 mL of blood clots were incubated overnight at 56 °C in 0.4 mL of proteinase K solution (1–10 U/mL proteinase K in 0.01 M Tris-HCl, pH 8, with 0.01 M EDTA and 0.5% sodium dodecyl sulfate). Subsequently, TE-saturated phenol (0.5 mL) was added. After vigorous mixing for 20 s, the samples were placed on ice for 10 min, followed by centrifugation at $16{,}000 \times g$ for 5 min at room temperature (20–25 °C). The aqueous layer was separated, mixed with 0.5 mL of 95–100% ethanol, and incubated at room temperature for 10 min. Following centrifugation at 12,000 rpm for 10 min, the DNA precipitate was collected and the supernatant was discarded. The DNA pellets were washed with 0.25 mL of 70% ethanol, dried, and dissolved in 20–200 µL of DNase-free water. The DNA samples were genotyped using the TaqMan® SNP genotyping assay system according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Statistical Analyses

Statistical analyses were performed using SAS9.4 TS Level 1M5 for Windows (SAS Institute, Cary, NC, USA). Statistical significance was set at $p < 0.05$. To approximate the normal distribution, IgG, IgM, and INF-γ, primary outcomes, were log-transformed. 1α, 25-(OH)_2 vitamin D level, the primary explanatory variable, was also log-transformed (Figure 2).
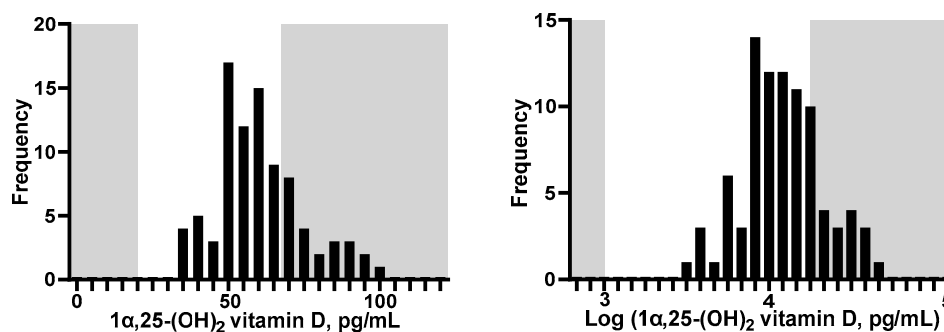

Figure 2. Histogram of 1α, 25-(OH)_2 vitamin D in the blood. Gray shading indicates an out-of-reference range.

2.6.1. Covariates

The following covariates suspected to be associated with immune response or vitamin D level were included in the statistical models: sex and age [21], rs671 (*ALDH2* gene variant) [19], height,

smoking status [22,23], alcohol intake [24,25], exercise habits [26,27], perceived stress [28], and medical history including allergic disease [29] and steroid use [30,31].

2.6.2. Mixed Model

Under the assumption of a linear regression between baseline blood levels of 1α, 25-(OH)$_2$ vitamin D and immune response to the COVID-19 mRNA vaccine, associations were estimated using mixed models that accounted for repeated measures, subpopulation as a random effect, and fixed effects of relevant covariates (proc mixed).

To further validate this association and explore non-linear relationships between vitamin D levels and immune responses, least squares geometric means and standard errors were calculated for three vitamin D quantiles (Q1: ≤52.4, Q2: 53.7–64, and Q3: ≥64.6). Mixed models for estimation included interactive terms between weeks post-vaccination (categorical) and categorical vitamin D levels.

3. Results

3.1. Baseline Characters of the Total Participants

Histograms of 1α, 25-(OH)$_2$ vitamin D concentration are shown in Figure 2. The vitamin D ranged from 33.7 and 99.8 pg/mL. None of the participants showed levels that fell below the general reference range for the Japanese population (20–60 pg/mL) [32], and 60.2% showed levels above the highest value within the reference range. Table 1 lists the baseline characteristics by tertile of 1α, 25-(OH)$_2$ vitamin D. Blood vitamin D levels were not associated with any of the variables. Four participants reported current smoking habits, and none of them had altered their smoking habits in the past year. Two participants had current steroid use, and the other participants had not reported steroid use in the previous 3 years: one participant reported current dyslipidemia and had the second-lowest vitamin D level of 37.3 pg/mL, while the other had no history of dyslipidemia in the past 3 years.

Table 1. Baseline characteristics of participants by vitamin D tertile.

	Q1	Q2	Q3	
1α, 25-(OH)$_2$ vitamin D, pg/mL	≤ 52.4	53.7–64.0	≥ 64.6	*p-value*
Sub-cohort 1, N	8	8	4	
Sub-cohort 2, N	14	15	13	
Sub-cohort 3, N	7	7	12	
Males, N (%)	13 (45%)	14 (47%)	16 (55%)	0.744[a]
Females, N (%)	16 (55%)	16 (53%)	13 (45%)	
Age				0.381[b]
Median	23	22.5	23	0.505[c]
IQR	(22–43)	(22–36)	(22–32)	
Body height, cm				0.990[b]
Median	166	164	165	0.801[c]
IQR	(160–170)	(156–172)	(161–169)	
Smoking status, yes, N (%)	2 (7%)	1 (3%)	1 (3%)	
Ethanol intake, N (%)				0.669[b]
<1 g/day	16 (55%)	17 (57%)	17 (59%)	
1–20 g/day	11 (38%)	12 (40%)	11 (38%)	
≥20 g/d	2 (7%)	1 (3%)	1 (3%)	
Exercise habit, N (%)				0.191[b]
No	11 (38%)	17 (57%)	7 (24%)	

<1 day/week	5 (17%)	2 (7%)	5 (17%)	
1–3 day/week	9 (31%)	8 (27%)	8 (28%)	
3 or more days/week	4 (14%)	3 (10%)	9 (31%)	
Perceived stress, N (%)				0.842 [a]
0 (no)	10 (34%)	12 (40%)	9 (31%)	
1 (unsure)	2 (7%)	3 (10%)	3 (10%)	
2 (mostly no)	6 (21%)	9 (30%)	5 (17%)	
3 (quite often)	9 (31%)	5 (17%)	10 (34%)	
4 (yes)	2 (7%)	1 (3%)	2 (7%)	
Steroid use, yes, N (%)	1 (3%)	0 (0%)	1 (3%)	0.586 [a]
Allergic disease, yes, N (%)	6 (21%)	11 (37%)	13 (45%)	0.151 [a]
Dyslipidemia, yes, N (%)	1 (3%)	0 (0%)	0 (0%)	0.659 [a]
ALDH2 rs671				0.817 [c]
*ALDH2*1/*1*	14 (48%)	14 (47%)	16 (55%)	
*ALDH2*1/*2*	10 (34%)	12 (40%)	11 (38%)	
*ALDH2*2/*2*	5 (17%)	4 (13%)	2 (7%)	

[a] Fisher's exact test, [b] Spearman's rank correlation, [c] Kruskal Wallis test.

3.2. Anti-S1 IgG Production

In the BNT162b2 group, anti-S1 IgG antibody titers peaked 2 weeks after the second dose (median of each tertile ranged from 1,118–2,383 BAU/mL, Table S1). In the mRNA-1273 group, the highest titers were in the first blood collection after the second dose (2680–3209 BAU/mL; Table S1). No consistent relationship was found between vitamin D and anti-S1 IgG levels in the comparison of medians.

Table 2 lists the results of the multivariate-adjusted regression model, including all observations in a single mixed model. When the model was adjusted for time course and vaccine type, a negative trend was observed for vitamin D and anti-S1 IgG levels (AIC = 1302, p = 0.15). With the addition of biological characteristics (sex, age, rs671, an East Asian-specific immune-related variant, and height), the fit improved (AIC = 1292), and a marginal and stronger negative association was estimated (p = 0.04). This trend was further strengthened in model 3, which included lifestyle and medical history (AIC = 1276, p = 0.0001). As shown in Table S2, this negative association was more robustly estimated in the sensitivity analyses that excluded one participant with dyslipidemia (p = 0.012, <0.001, and < 0.001 for models 1, 2, and 3, respectively).

Table 2. Estimated fixed effects of baseline characteristics on log-transformed anti-S1 IgG (BAU/mL).

	Model 1		Model 2		Model 3	
	N = 88		N = 88		N = 88	
	651 observations		651 observations		651 observations	
	AIC = 1302		AIC = 1292		AIC = 1276	
	β	*p-value*	β	*p-value*	β	*p-value*
BNT162b2 (reference)						
mRNA-1273	0.783	0.015	0.851	<0.001	0.860	<0.001
Age (per year old)			-0.009	0.001	-0.012	<0.001

Female sex		0.043	0.608	-0.054	0.550
ALDH2 variant allele number		-0.134	<0.001	-0.186	<0.001
Height (per cm)		-0.011	0.030	-0.012	0.021
Smoking status, yes				0.291	0.011
Ethanol intake (per category)				-0.062	0.196
Exercise habit (per category)				-0.037	0.120
Perceived stress (per category)				0.047	0.021
Steroid use, yes				-0.140	0.443
Allergic disease, yes				0.024	0.681
Dyslipidemia, yes				-1.013	<0.001
Log (vitamin D* pg/mL)	-0.166	0.154	-0.238	0.044	-0.474	<0.001

β, partial correlation coefficient. AIC, Akaike's Information Criterion. *1α, 25-$(OH)_2$ vitamin D.

Next, to depict the non-linear relationship between vitamin D and anti-S1 IgG levels, we estimated the adjusted means of IgG values for the three quantile groups of vitamin D. As shown in Figure 3a, the highest IgG levels were consistently observed in the Q1 group (the lowest vitamin D level) following the second dose. The orders of the Q2 and Q3 groups were inconsistent.
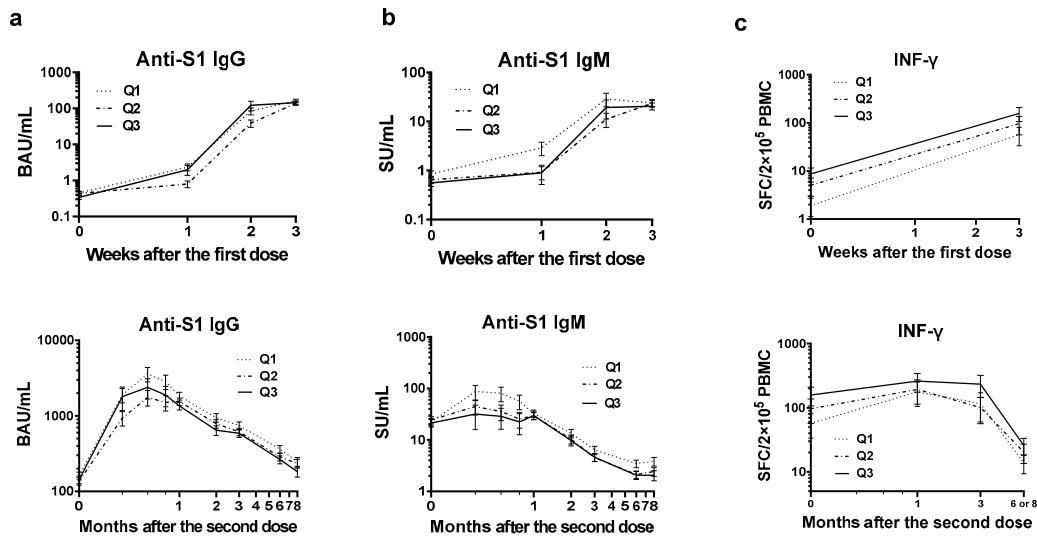

Figure 3. Adjusted means of antibody levels and INF-γ-producing cell counts by baseline blood 1α, 25-$(OH)_2$ vitamin D levels. The participants were categorized into three groups based on the baseline blood 1α, 25-$(OH)_2$ vitamin D levels, ≤ 52.4, 53.7–64.0, and ≥ 64.6, for Q1 (N = 29), Q2 (N = 30), and Q3 (N = 29). Least-squares geometric means and standard errors were determined using mixed models, accounting for repeated measures and random effects of the sub-cohort. The models included all the covariates presented in Table 1: vaccine type, number of weeks (categorical variables), and vitamin D groups (Q1–3) × time course (interaction term) as fixed effects. Q1, INF-γ represents SARS-CoV-2 Spike protein-specific IFN-γ producing cell counts.

3.3. Anti-S1 IgM Production

Anti-S1 IgM peak was observed 1 week after the second dose in the BNT162b2 group (median for each of the three quantiles ranged from to 22–63 SU/mL), 1 week earlier than IgG, although it was not observed in the mRNA-1273 group due to lack of frequent sampling. No consistent relationship was found between the quantile order of vitamin D and the median IgM titers (Table S3).

As shown in Table 3, model 1 estimated a trend toward a negative correlation between IgM and vitamin D levels (p = 0.196, AIC = 1753). The addition of biological characteristics gave a stronger negative estimate with a lower *p-value* (0.037) with a better model fit (AIC = 1749, model 2), and the further inclusion of lifestyle and medical history resulted in the best-fitting model (AIC = 1724), estimating the strongest association with the lowest *p-value* (< 0.001) among these models. As listed in Table S4, sensitivity analyses that excluded one participant with dyslipidemia estimated robust negative correlations with low *p-values* (0.004, < 0.001, and < 0.001 for models 1, 2, and 3, respectively).

Table 3. Estimated fixed effects of baseline characteristics on log-transformed anti-S1 IgM (SU/mL).

	Model 1 N = 88 651 observations AIC = 1753		Model 2 N = 88 651 observations AIC = 1749		Model 3 N = 88 651 observations AIC = 1724	
	β	*p-value*	β	*p-value*	β	*p-value*
BNT162b2 (reference)						
mRNA-1273	-0.026	0.906	0.080	0.454	0.036	<0.001
Age (per year old)			-0.015	<0.001	-0.012	0.021
Female sex			-0.173	0.153	-0.249	0.011
ALDH2 variant allele number			0.046	0.387	-0.013	0.196
Height (per cm)			-0.016	0.023	-0.019	0.120
Smoking status, yes					0.016	0.021
Ethanol intake (per category)					0.044	0.443
Exercise habit (per category)					0.023	0.681
Perceived stress (per category)					-0.002	<0.001
Steroid use, yes					-0.884	<0.001
Allergic disease, yes					0.106	<0.001
Dyslipidemia, yes					-1.733	<0.001
Log (vitamin D* pg/mL)	-0.215	0.196	-0.352	0.037	-0.675	<0.001

β, partial correlation coefficient. AIC, Akaike's Information Criterion. *1α, 25-(OH)$_2$ vitamin D.

The non-linear vitamin D—IgM relationship is depicted in Figure 3b by the quantiles of vitamin D. The highest IgM levels were consistently observed in the Q1 group (lowest vitamin D levels) following the second dose.

3.4. SARS-CoV-2 Spike Protein-Specific INF-γ Producing Cell Counts

Repeated observation of ELISPOT counts of SARS-CoV-2 spike protein-specific INF-γ-producing cells was analyzed for 26 participants, with 122 observations in sub-cohort 3 and 19 participants with 19 observations in sub-cohort 2. The highest median values in the Q1-3 groups were observed for Q1 and Q3 (Table S5).

As listed in Table 4, model 1 showed no effect on baseline vitamin D levels ($p = 0.48$, AIC = 420). Although the model fit worsened in model 2 (AIC = 427), including biological characteristics revealed a positive effect of vitamin D ($p = 0.036$). Model 3 showed a stronger positive effect of vitamin D with a lower probability of α error ($p = 0.003$, AIC = 426).

The non-linear relationship between vitamin D and ELISPOT is illustrated in Figure 3c by estimating the corresponding adjusted means for each time course by the three quantile groups. The highest values were consistently estimated for Q3, which had the highest vitamin D.

Table 4. Estimated fixed effects of baseline characteristics on log-transformed on IFN-γ ELISPOT count.

	Model 1 N = 45 141 observations AIC = 420		Model 2 N = 45 141 observations AIC = 427		Model 3 N = 45 141 observations AIC = 426	
	β	*p-value*	β	*p-value*	β	*p-value*
BNT162b2 (reference)						
mRNA-1273	-3.260	<0.001	-3.610	<0.001	-3.796	<0.001
Age (per year old)			0.010	0.141	0.013	0.083
Female sex			0.469	0.112	0.771	0.022
ALDH2 variant allele number			0.345	0.012	0.465	0.002
Height (per cm)			0.014	0.436	0.013	0.485
Smoking status, yes					-0.632	0.283
Ethanol intake (per category)					0.313	0.082
Exercise habit (per category)					0.084	0.360
Perceived stress (per category)					-0.014	0.853
Steroid use, yes					0.460	0.578
Allergic disease, yes					-0.321	0.132
Log (vitamin D* pg/mL)	0.252	0.479	0.838	0.036	1.277	0.003

β, partial correlation coefficient. AIC, Akaike's Information Criterion. *1α, $25-(OH)_2$ vitamin D. To enhance the mixed models, the observations 6 and 8 months following the second dose were categorized under the same label.

4. Discussion

To the best of our knowledge, this study is the first to report the effects of baseline blood levels of active vitamin D, 1α, $25-(OH)_2$ vitamin D, on humoral and cellular immune reaction to COVID-19

mRNA vaccination. The participants showed high vitamin D levels, from normal to above the reference range, within which vitamin D levels were negatively associated with humoral immunity and positively with cellular immunity.

Active vitamin D is a ligand of a nuclear receptor found in immune cells, such as dendritic cells, macrophages, and T and B cells [33]. After transmigration into the nucleus, activated vitamin D receptors bind to vitamin D response sequences in the genomic DNA and regulate the expression of various target genes, including cell cycle regulators [24,34]. Active vitamin D suppresses the proliferation of B cells and the production of IgG and IgM [35,36], consistent with the findings in this study. Since active vitamin D increases the expression of p27 in B lymphocytes and reduces the mRNA levels of cell division growth factors, such as cyclin-dependent kinases 4 and 6 and cyclin D [37], high levels of blood active vitamin D may result in the inhibition of B cell proliferation, potentially suppressing antibody production, as observed in this study.

Active vitamin D suppresses Th1-mediated inflammatory responses [35,38] and Th1 are the primary cells to produce INF-γ in response to antigen exposure [39]. However, we found consistently higher counts of INF-γ-producing cells in the Q3 group, which had the highest levels of active vitamin D. One hypothesis to bridge this gap may be the recent finding regarding memory T cells in human intervention studies; Saroha et al. supplemented the intervention group with precursor vitamin D [7], confirmed an increase in active 1α, 25-(OH)$_2$ vitamin D concentration in supplemented group, and found upregulations in the expression of memory T cell-related genes through comprehensive analysis of PBMC mRNA. The hypothesis that vitamin D favors the preservation of memory T cells but not the proliferation of effector T cells may clarify why we did not observe a notable vaccine-induced increase in the INF-producing cell count linked to vitamin D in our study. We observed slightly higher cell counts, which were consistently estimated in Q3 from the baseline.

Lavell et al. reported no association between baseline precursor vitamin D and SARS-CoV-2 Spike protein-specific INF-γ release [8,37]. These results do not contradict our findings, because the concentrations of vitamin D in the precursor and active forms are not proportional [40]. In addition, since the ELISPOT assay used in our study is an advanced technology developed to increase sensitivity [41,42], the ELISA used in their study might not have been sensitive enough to detect the effects.

One of the greatest challenges of COVID-19 vaccines is breakthrough infections caused by immune escape due to mutations in the receptor-binding domain (RBD) of the spike protein, which reduces the affinity between the antibody and the RBD [43]. In contrast, cell-mediated immunity is less susceptible to mutations in viral strains as T-cell receptors recognize the epitopes of polyclonal spike proteins and RBDs [44]. Maintaining a high concentration of active vitamin D in the blood and a strong cellular immune system may contribute to the long-term reduction of severe COVID-19.

This study has some limitations. First, the small number of participants limited statistical power. Although we adopted a mixed model accounting for repeated measurements and sub-cohorts to minimize the problem, there may be associations that could not be detected. In particular, the evaluation of cellular immunity was largely limited by research funding (141 observations from 45 participants). Second, the lowest active vitamin D level (33.7 pg/mL) in the current cohort was above the lowest reference range (20 pg/mL). Active vitamin D levels below 20 pg/mL have been shown to increase the incidence of severe illness and mortality [45-48]. We could not verify the effects on the immunogenicity of the COVID-19 vaccine. Third, the extent to which the immunogenicity effects observed in this study influenced actual COVID-19 infections. To further elucidate the role of vitamin D in enhancing vaccine-induced immunity, a longitudinal cohort study with a larger number of participants will be required.

5. Conclusions

The active form of vitamin D, 1α, 25-(OH)$_2$ vitamin D, may suppress increased antibody titers while promoting cellular immune responses following COVID-19 mRNA vaccination. Maintaining blood levels of active vitamin D above the reference range could reduce the risk of breakthrough infections and severe disease by enhancing cellular immunity.

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