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ORIGINAL RESEARCH

Vitamin D Binding Protein: A Potential Factor in Geriatric COVID-19 Acute Lung Injury

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Background: Previous research indicated that vitamin D binding protein (VDBP) is an independent multifunctional protein that plays a vital role in acute inflammatory and tissue damage. However, its role in acute lung injury (ALI) due to coronavirus disease 2019 (COVID-19) is unclear, and studies are lacking. This study intends to investigate the difference in serum VDBP levels in COVID-19 patients with ALI or without ALI and further explore the role of VDBP in the inflammatory response of ALI through cellular models.

Methods: The serum was collected from COVID-19 patients, and the concentration of serum VDBP was detected. Construct a VDBP gene-silencing plasmid and transfect it into human alveolar epithelial A549 cells. After 72 hours of lipopolysaccharide (LPS) intervention, The inflammatory factors interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were detected, and cell counting kit-8 (CCK-8) assay was used to detect cell viability. Flow cytometry was used to detect cell apoptosis.

Results: The serum concentration of VDBP was significantly higher in COVID-19 with ALI ($P < 0.05$). Correlation analysis indicated serum VDBP positively correlated with leukocyte (r=0.329, $P = 0.002$), c-reaction protein ($r = 0.470$, $P < 0.001$), serum amyloid A ($r = 0.900$, $P < 0.001$), procalcitonin ($r = 0.670$, $P < 0.001$), and interleukin 6 ($r = 0.452$, $P < 0.001$). Simultaneously, the logistic regression analysis showed that increased serum VDBP was an independent risk factor for ALI in COVID-19 patients (OR 1.003 95% CI 1.001–1.006, $P = 0.002$). In human alveolar epithelial A549 cells, after LPS intervention, the inflammatory factor IL-1β and TNF-A significantly reduced in the VDBP gene silencing group compared to the negative control (NC) group ($P < 0.05$). The cell viability of the VDBP gene silencing group was significantly increased compared to the NC group, and the cell apoptosis rate was significantly reduced ($P < 0.05$).

Conclusion: In COVID-19 patients, acute lung injury may lead to increased serum concentration of VDBP. VDBP plays a vital role in promoting inflammatory response and apoptosis of bronchial epithelial cells.

Keywords: Vitamin D binding protein, COVID-19, acute lung injury, pathogenesis

Introduction

Acute lung injury (ALI) is the most common factor contributing to the increasing mortality of patients, which not only seriously affects the quality of life of surviving patients but also imposes a heavy economic burden on society.[1] Since December 2019, the coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread the world. COVID-19 has generated over 770 million confirmed cases, and over 6.9 million deaths have been reported globally, significantly impacting human health.[2]

Although the world pandemic COVID-19 has ended, the SARS-CoV-2 has never disappeared, and SARS-CoV-2 infections are ongoing. The pathogenesis of ALI due to SARS-CoV-2 is complex, and the replication of large quantities of the virus, the release of a storm of pro-inflammatory cytokines, the initiating chemokine responses, and the infiltration of inflammatory cells all play essential roles.[3] Due to the incomplete elucidation of its pathogenesis and the lack of effective indicators for early diagnosis and treatment, further research is needed to elucidate its pathogenesis and search for new clinical treatment targets.

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Previous research indicated that Vitamin D deficiency was closely associated with the severity and mortality of COVID-19 and increased the incidence rate of acute respiratory failure.[4–6] Nevertheless, Domazet J. found no benefit in vitamin D supplementation to patients with severe COVID-19 admitted to the ICU and in need of respiratory support.[7] Vitamin D-binding protein (VDBP), which was well known as the primary carrier of vitamin D metabolites, was also found to be closely related to COVID-19 in recent research.[8–17] The association between genetic polymorphism of VDBP and COVID-19 may depend on the regulatory pleiotropic effects of biologically available vitamin D levels.[18] In addition, research has found that VDBP concentration is not affected by vitamin D levels, but estrogen, glucocorticoids, and inflammatory cytokines regulate it.[19] Although data on VDBP and COVID-19 are limited, published studies have shown higher levels of VDBP in patients with severe COVID-19, and serum VDBP levels were positively correlated with mortality and days of hospitalization.[14] Meanwhile, some researchers found that VDBP was also detected in bronchoalveolar lavage fluid of ARDS patients.[20]

VDBP not only plays a vital role in maintaining stable serum levels of vitamin D and modulating its bioavailability but also plays an essential role in the progress of inflammatory and immunoregulation functions independent of vitamin D carriage.[21] VDBP is synthesized in the liver. Only 5% of total plasma VDBP is bound and transports vitamin D and metabolites to the target organs. The remaining 95% is found in different organs (heart, brain, lungs, kidneys, spleen, tests, and uterus), where various functions are performed, such as increased neutrophil chemotaxis, macrophage activation, actin clearance, fatty acid transport, enhanced the chemotactic activity of the complement peptide C5a, and activity as VDBP-macrophage activating factor (DBP-MAF).[21–23]

Therefore, VDBP, an independent multifunctional protein, is essential in acute inflammatory and tissue damage. However, its role in ALI due to COVID-19 is unclear, and studies are lacking. This study intends to investigate the difference in serum VDBP levels in COVID-19 patients with ALI and further explore the role of VDBP in the inflammatory response and apoptosis of ALI through cellular models.

Materials and Methods

Study Design

As a prospective cross-sectional observational study, we collected the COVID-19 patients in the Department of Geriatrics of the Shandong Provincial Hospital Affiliated to Shandong First Medical University from December 1, 2022, to September 30, 2023.

Inclusion criteria: Age $\geqslant$ 65 years. Patients diagnosed with COVID-19 through symptoms, polymerase chain reaction tests, and other clinical examinations.

Exclusion criteria: Patients with a history of severe chronic respiratory diseases, chronic liver disease, congestive heart failure, and malignant tumors. Patients with severe liver and kidney dysfunction, patients with immune system disorders, and coagulation dysfunction. Patients with acute cerebrovascular disease, acute myocardial infarction, and acute pulmonary embolism, and patients with a history of surgical trauma within the past six months.

The enrolled patients (n=208) were divided into two groups: COVID-19 with ALI group (n=56) and COVID-19 without ALI group (n=152), based on whether ALI was present according to the Berlin Definition:[24] Respiratory symptoms onset or new or worsening within a week; Chest imaging (X-ray or CT scan) showing bilateral opacities, not fully explained by effusions, lobar/lung collapse, or nodules; Respiratory failure not fully explained by cardiac failure or fluid overload; PaO2/FiO2 $\leq$ 300mmHg.

Written informed consent was obtained from each participant. We confirm that our study complies with the Declaration of Helsinki. The Shandong Provincial Hospital Affiliated to Shandong First Medical University ethics committee approved this study (SZRJJ: No. 2021–411, approval date: November 25, 2021).

Data and Serum Collection

Age, gender, height, weight, comorbidities, and length of stay (LOS) were collected. Routine blood tests, C-reaction protein (CRP), serum amyloid A (SAA), procalcitonin (PCT), and interleukin-6 (IL-6) were obtained from patients' routine hospital laboratory records upon admission to the ward.

Collect peripheral blood samples from the above subjects within 48 hours after admission. The serum separation was achieved through 15 minutes' centrifugation at 1000×g and then storage at −80°C.

Detection of Serum VDBP and 25(OH)D Levels

The serum concentration of VDBP and 25(OH)D were measured using enzyme-linked immunosorbent assay kit (ABclonal, US) and 25-Hydroxy vitamin D RIA kit (DiaSorin. Stillwater, Minnesota), respectively, following standard protocols.

Cell Culture

Human alveolar epithelial A549 cells were purchased from FuHeng Cell Center (Shanghai, China) and were cultured in DMEM medium (GIBCO, US) containing 10% fetal bovine serum, placed in a 5% CO_2, saturated humidity, 37 °C incubator, observed the cell growth condition, replaced with a new culture medium every other day, and passed on when the cells adhered to the wall and grew to 80%. Cells in the logarithmic proliferation phase were taken for subsequent experiments.

VDBP shRNA Transfection of A549 Cells

For the silencing experiments, alveolar epithelial cells A549 were seeded in 96-well plates (2×10^4 cell/well) and transfected the next day, according to the manufacturer's instruction, with VDBP shRNA (Genechem, Shanghai, China) (CCTGTGAAAGTAATTCTCCAT; CCAAAGGAATATGCTAATCAA; CTCAGCAATCTCATAAAGTTA). After 48 hours of transfection, the knockout of VDBP expression in transfected cells was confirmed through qPCR analysis and recorded as the shRNA-VDBP group. The Universal scrambled negative control shRNA duplex was used as the negative control (shRNA-NC group). Then, the culture was done with a cell culture medium containing 200 ug/mL LPS for 48 hours, and the cells were recorded as the shRNA-VDBP+LPS group and the shRNA-NC+LPS group, respectively.

Quantitative Real-Time PCR (qPCR)

The alveolar epithelial cells A549 were cultured in a cell culture medium containing 200 ug/mL LPS for 48 hours. According to the manufacturer's instructions (Cwbio, China), total RNA was isolated using a TRIZOL reagent. The separated mRNA was reverse transcribed through the HiFiScript cDNA Synthesis Kit (Cwbio, China). Amplify using specific primers (VDBP F 5-GTGGCACGTTTGAACAGGTC-3'; R 5-CTTTCGTTCCAGGCCCTCTT-3') on the quantitative real-time PCR instrument (Hehui Biotechnology H9800), β-action as internal control (β-actin F 5'-AGACCTGTACGCCAACACAG-3' R 5'- CGGACTCGTCATACTCCTGC-3') with the $2^{-\Delta\Delta Ct}$ method for analyzing the relative expression of genes.

Western-Blot Assay

Collect cells from each group, add an appropriate amount of lysate and protease inhibitors, and extract protein samples. The protein concentration was measured using the BCA method. Then separate equal amounts of proteins on 12% SDS-PAGE for Western blot using antibodies against VDBP (1:2000 dilution, Proteintech, US) and GAPDH (1:5000 dilution, Proteintech, US), the protein was observed using the ECL chemiluminescence method. GAPDH was used as an internal reference. Analyze grayscale values using image J.

ELISA Assay for IL-1β, TNF-α

According to the manufacturer's instructions, IL-1β and TNF-α in cell supernatant were measured using the ELISA kits (ABclonal, US).

Cell Proliferation Assay (CCK8)

After the transfection of cells, cell viability was measured by the CCK-8 method (MedChemExpress, US) on 0–4 days in each group of cells. The optical density was assessed with a microplate reader (Tecan F50, Switzerland) at a wavelength of 450 nm.

Flow Cytometry for Apoptosis Analysis

Collect cells and centrifuge them. Add 100 μL 1× binding buffer to resuspend cells, then add in sequence 5μL annexin V and 10μL PI and incubate at room temperature in the dark for 15 minutes before adding 400μL binding buffer. Finally, flow cytometry (FACSCalibur, BD, USA) was used to detect cell apoptosis.

Statistical Analysis

Statistical analysis was performed using SPSS version 28.0 (IBM-SPSS, Armonk, NY, USA). Use the Shapiro–Wilk test for the normal distribution test. The normal distribution data was presented by mean ± standard deviation (SD), compared using the ANOVA test, and within the group using the Bonferroni method. Non-normally distributed data were shown as median (interquartile range, IQR), and the Kruskal–Wallis test was used for comparison. The categorical variables were expressed by frequency (composition ratio or percentage) and compared by the chi-square test.

Pearson correlation (normal distribution data) or Spearman correlation (nonnormal distribution data) was used to assess the relationship between serum VDBP and the disease-related inflammatory indicators. Logistic regression analysis was used to analyze the correlation between the VDBP and the prognosis of COVID-19. Receiver operating characteristic (ROC) curves were constructed, and the area under the curves (AUCs) was determined to detect the prognosis of COVID-19. A p-value < 0.05 (two-tailed) was considered statistically significant.

Results

Clinical Characteristics of the Subjects

The study included 208 COVID-19 subjects, 73% (n=152) patients without ALI and 27% (n=56) patients with ALI. There was no significant difference between the two groups in age, sex, body mass index (BMI), and comorbidity ($P > 0.05$). However, the mortality and length of stay (LOS) of the ALI group were significantly higher ($P < 0.001$, Table 1). Regarding disease-related inflammatory indicators, the ALI group's WBC, CRP, SAA, PCT, and IL-6 were significantly higher than those without ALI ($P < 0.05$, Table 1).

Table 1 Characteristics of the Included Subjects

Characteristics	COVID-19 Without ALI (n = 152)	COVID-19 With ALI (n = 56)	P Value
Age, year (mean ± SD.)	83.4 ± 7.9	86.0 ± 6.9	0.202
Sex (man, %)	108 (71.1)	32 (57.1)	0.055
BMI, kg/m^2 (mean ± SD)	24.6 ± 3.3	23.9 ± 6.9	0.928
Comorbidity, n (%)			
Diabetes	46 (30.3)	14 (25.0)	0.871
Hypertension	82 (53.9)	32 (57.1)	0.820
Coronary heart disease	76 (50.0)	24 (42.9)	0.565
Arrhythmias	24 (15.8)	4 (7.1)	0.323
Nervous system disease [a]	64 (42.1)	32 (57.1)	0.161
Chronic pulmonary disease [b]	12 (7.9)	12 (21.4)	0.083
Mortality, n (%)	10 (6.6)	34 (60.7)	< 0.001
Length of stay, day (mean ± SD.)	15.2 ± 11.1	25.1 ± 18.1	0.002
Inflammatory indicators			
WBC (10^9/L, mean ± SD)	6.48 ± 3.52	8.51 ± 5.07	0.042
CRP [mg/mL, median (IQR)]	14.61 (4.95, 47.10)	75.01 (45.57, 128.2)	< 0.001
SAA [mg/mL, median (IQR)]	51.28 (9.06, 204.41)	404.50 (101.56, 660.68)	< 0.001
PCT [ng/mL, median (IQR)]	0.06 (0.04, 0.15)	0.18 (0.08, 0.69)	< 0.001
IL-6 [pg/mL, median (IQR)]	9.44 (2.30, 26.22)	31.45 (8.71, 103.78)	0.003

Notes: [a]Nervous system disease: cerebrovascular disease, Parkinson's disease, Alzheimer's disease; [b]Chronic pulmonary disease: asthma, chronic obstructive pulmonary disease, interstitial lung disease.
Abbreviations: BMI, body mass index; WBC, white blood cell; CRP, C-reaction protein; SAA, Serum amyloid A; PCT, procalcitonin; IL-6, interleukin-6.

The Serum Concentrations of VDBP and 25OHD

The serum concentrations of VDBP in COVID-19 patients with ALI was 1540.37 ± 403.10 ng/mL, significantly higher than those without ALI (1183.37 ± 225.85 ng/mL, $P < 0.001$). However, there was no significant difference in serum 25OHD levels between COVID-19 patients with ALI and those without ALI (37.9± 19.8 ng/mL vs 43.6± 25.2 ng/mL, $P > 0.05$).

Correlation Analysis and Regression Analysis of VDBP

Correlation analysis indicated serum VDBP was positively correlated with WBC ($r = 0.329$, $P = 0.002$, Figure 1A), CRP ($r= 0.470$, $P < 0.001$, Figure 1B), SAA ($r = 0.900$, $P < 0.001$, Figure 1C), PCT ($r = 0.670$, $P < 0.001$, Figure 1D), and IL-6 ($r = 0.452$, $P < 0.001$, Figure 1E). However, there was no association between serum VDBP and LOS ($P = 0.469$, Figure 1F). After excluding the effects of age, gender, comorbidities, mortality rate, LOS, 25(OH)D, and inflammatory indicators in Table 1, logistic regression analysis showed serum VDBP was an independent risk predictor of ALI in COVID-19 patients (OR 1.003, 95% CI 1.001–1.006, $P = 0.002$) and mortality risk (OR 1.002, 95% CI 1.001–1.004, $P = 0.01$).

The ROC curve showed that VDBP > 1265 ng/mL was a marker to judge the risk of ALI in COVID-19, with a sensitivity of 84.00% and specificity of 73.85% (AUC 0.803, 95% CI 0.690–0.917, $P < 0.001$, Figure 2A). At the same time, the ROC curve showed that VDBP > 1270 ng/mL was a marker to judge the risk of mortality from COVID-19, with a sensitivity of 78.95% and specificity of 69.01% (AUC 0.755, 95% CI 0.613–0.898; $P < 0.001$, Figure 2B).

Significant Overexpression of VDBP in LPS-Induced Injury of Alveolar Epithelial Cells

Western blot Results showed that the expression of VDBP in alveolar epithelial cells A549 was significantly increased compared to the negative control (NC) group after being cultured in a cell culture medium containing 200 ug/mL LPS for 24–72 hours ($P < 0.05$, Figure 3A). The q-PCR results also showed a significant increase in VDBP gene expression in the LPS group compared to the NC group ($P = 0.0025$, Figure 3B).

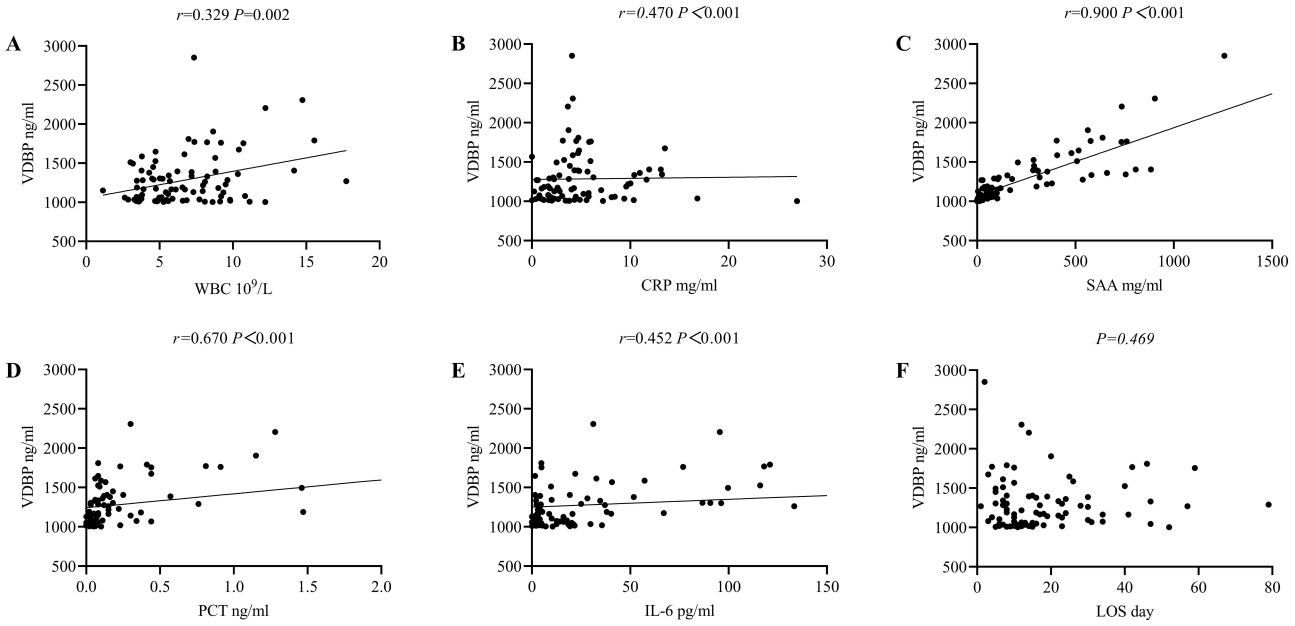

Figure 1 The correlation between serum VDBP and inflammatory indicators and length of stay. (**A**) The correlation between VDBP and WBC. (**B**) The correlation between VDBP and CRP. (**C**) The correlation between VDBP and SAA. (**D**) The correlation between VDBP and PCT. (**E**) The correlation between VDBP and IL-6. (**F**) The correlation between VDBP and LOS. WBC: white blood cell, CRP: C-reaction protein, SAA: Serum amyloid A, PCT: procalcitonin, IL-6: interleukin-6, LOS: length of stay.

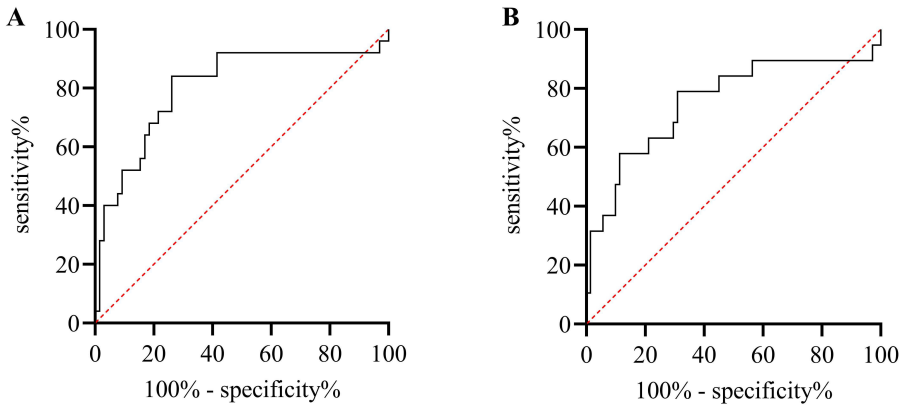

Figure 2 Receiver operating characteristic curves for the VDBP to detect the prognosis of COVID-19. (**A**) Receiver operating characteristic (ROC) curves for the VDBP to detect the ALI of COVID-19. (**B**) ROC curves for the VDBP to detect the mortality of COVID-19.

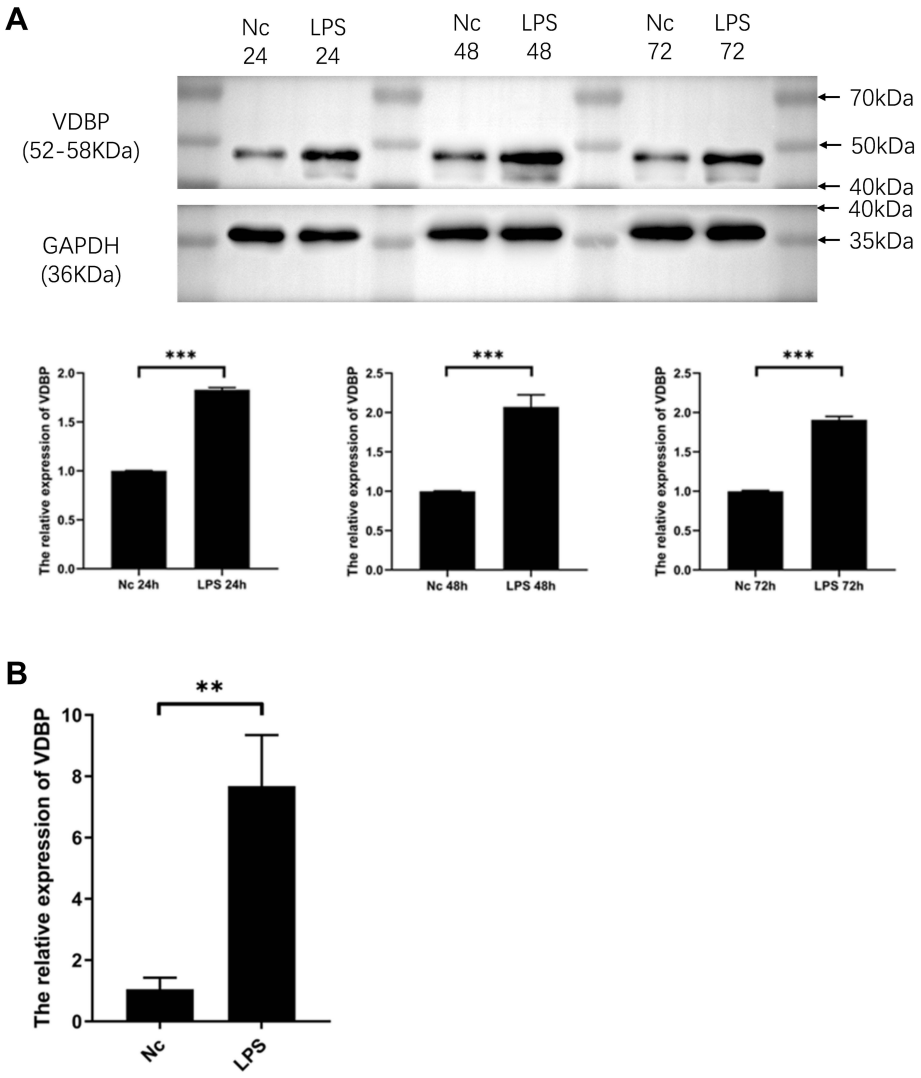

Figure 3 The VDBP expression in injured alveolar epithelial cells. (**A**) The VDBP protein level in LPS-induced injury of alveolar epithelial cells at different time points. The representative Western blot results and the relative expression of VDBP from the Western blot were shown. (**B**) The VDBP gene expression in the LPS-induced injury of alveolar epithelial cells. The q-PCR was used to analyze the relative expression of genes. Columns and error bars represented mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$. VDBP: Vitamin D-binding protein, LPS: Lipopolysaccharide, NC: negative control, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

The Effect of VDBP Gene Silencing on the Inflammatory Factors in LPS-Induced Injury of Alveolar Epithelial Cells

We further explore the role of VDBP in ALI through silencing experiments. The ELISA results showed that the expression of inflammatory factors IL-1β and TNF-α in alveolar epithelial cells significantly increased after 48 hours of cultivation in the cell culture medium containing 200 ug/mL LPS, while the VDBP gene silencing group significantly reduced the levels of IL-1β (Figure 4A), TNF-α (Figure 4B) compared to the NC group after LPS intervention, whether in the supernatant or cells ($P < 0.05$).

The Effect of VDBP Gene Silencing on the Cell Viability of LPS-Induced Injury of Alveolar Epithelial Cells

We evaluated the effect of VDBP gene silencing on the cell viability of the LPS-induced jury of alveolar epithelial cells using the CCK8 method. After 48 hours of cultivation in the cell culture medium containing 200 ug/mL LPS, the cell viability of alveolar epithelial cells A549 was significantly inhibited ($P< 0.05$). However, the cell viability (96h) of the VDBP gene silencing group was significantly improved compared to the NC group ($P = 0.001$, Figure 5).

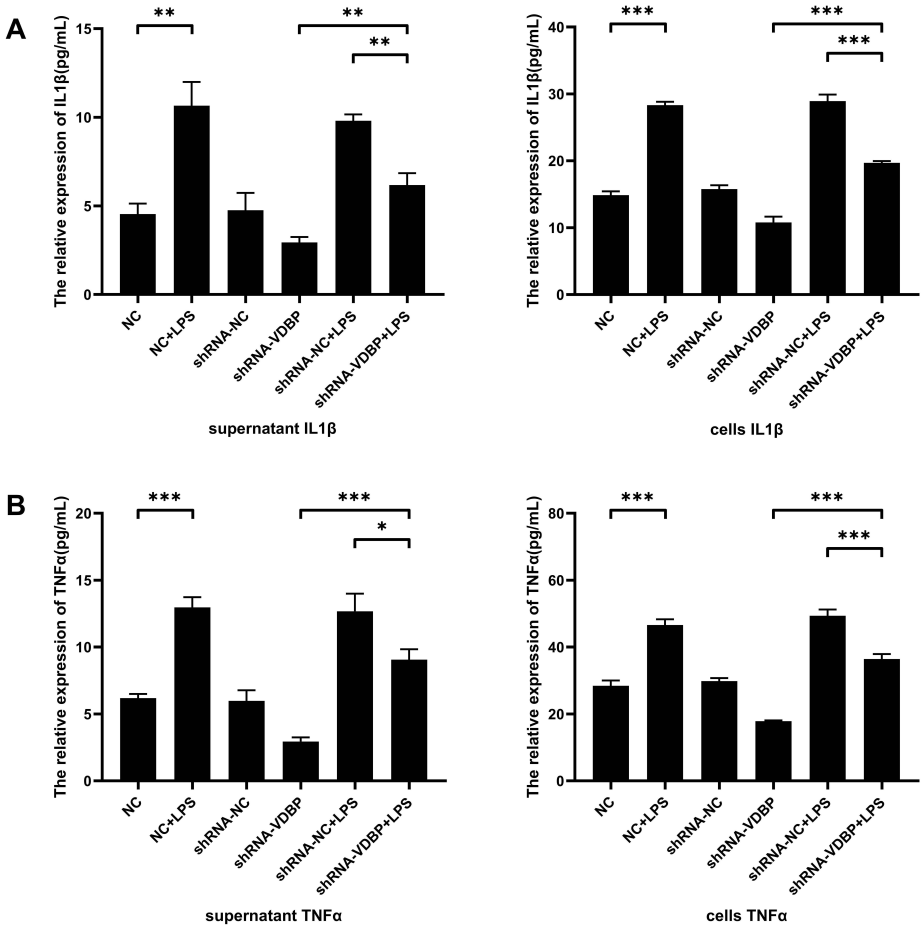

Figure 4 VDBP gene silencing affects the inflammatory factors of injured alveolar epithelial cells. (**A**) Enzyme-linked immunosorbent assay (ELISA) was conducted to detect IL-1β in alveolar epithelial cells and supernatant. (**B**) ELISA was conducted to detect TNF-α in alveolar epithelial cells and supernatant. Columns and error bars represented mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. VDBP: Vitamin D-binding protein, LPS: Lipopolysaccharide, NC: negative control.

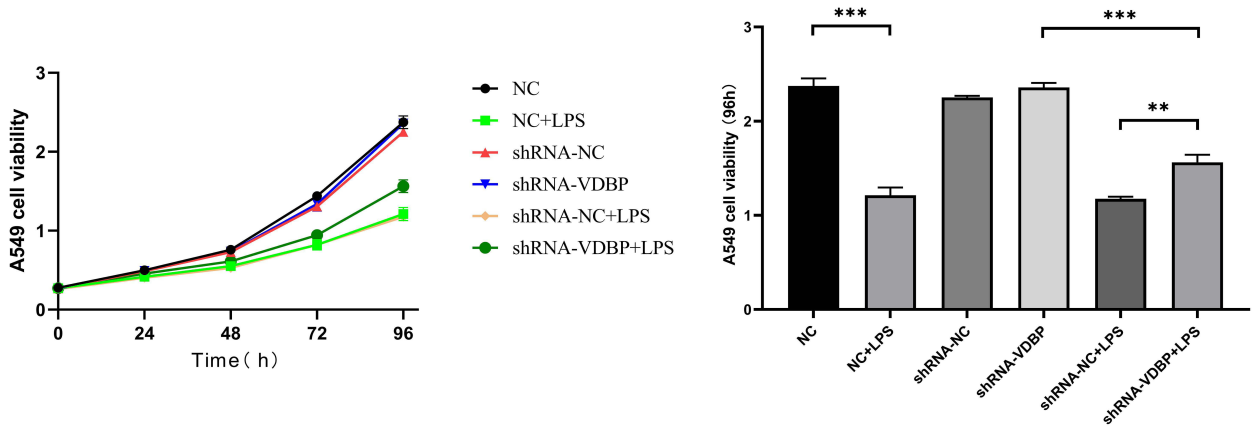

Figure 5 VDBP gene silencing affects the viability of injured alveolar epithelial cells. Cell viability was assessed with CCK-8 assay. Columns and error bars represented mean ± SEM. ***P* < 0.01, ****P* < 0.001. VDBP: Vitamin D-binding protein, LPS: Lipopolysaccharide, NC: negative control.

The Effect of VDBP Gene Silencing on LPS-Induced Apoptosis of Alveolar Epithelial Cells

Flow cytometry showed that the apoptosis rate of alveolar epithelial cells A549 significantly increased after LPS intervention. In contrast, the VDBP gene silencing group showed a significant decrease in cell apoptosis rate compared to the control group ($P = 0.002$, Figure 6).

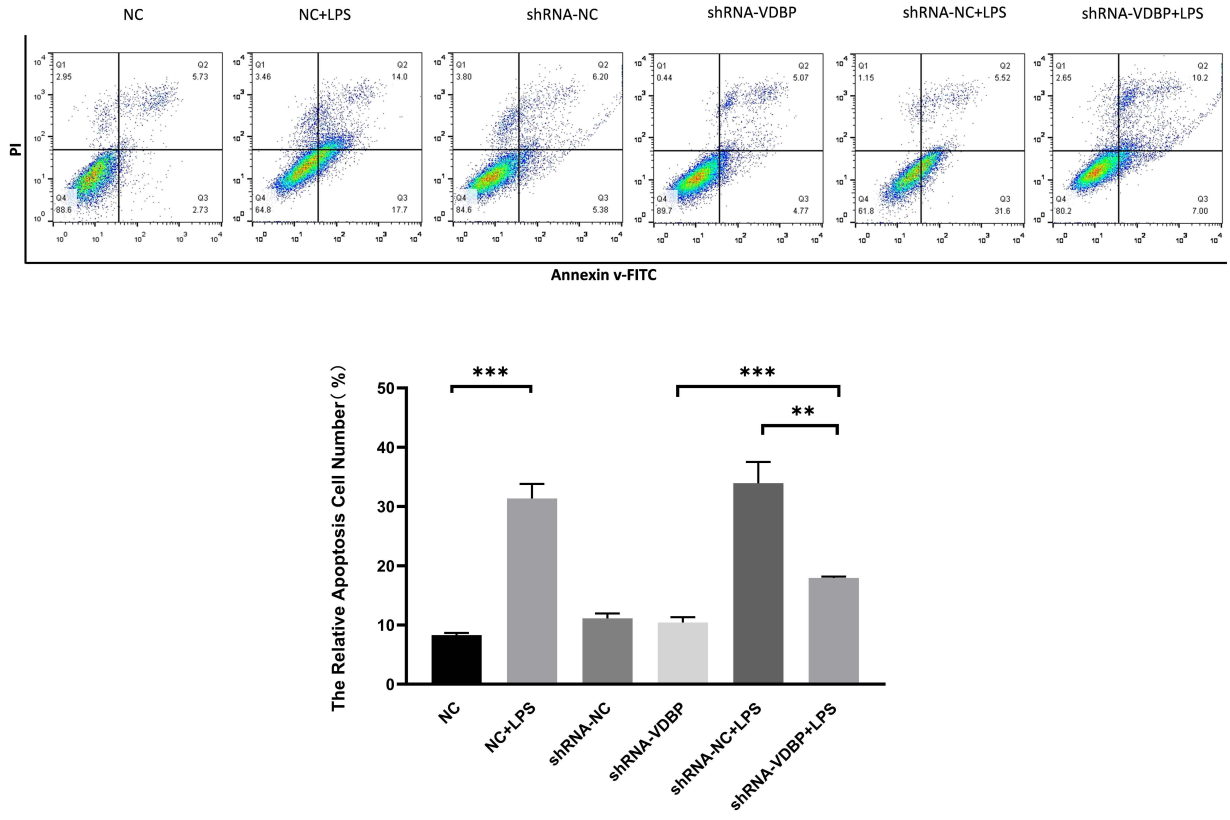

Figure 6 VDBP gene silencing affects the apoptosis of injured alveolar epithelial cells. Flow cytometry showed the apoptosis rate of alveolar epithelial cells. Representative and quantitative flow cytometry results were shown. Columns and error bars represented mean ± SEM. ***P* < 0.01, ****P* < 0.001. VDBP: Vitamin D-binding protein, LPS: Lipopolysaccharide, NC: negative control.

Discussion

Since 2019, COVID-19 has swept the world, causing many deaths due to ALI. Research on its risk factors and prognosis is proliferating, and many studies have confirmed the role of VitD in COVID-19.[25,26] Some scholars gradually found that the VDBP also played a crucial role in COVID-19. Previous studies have found that the VDBP has independent biological functions, including macrophage activation, fatty acid transport, neutrophil chemotaxis, and actin clearance.[19,27,28] Although VDBP plays a vital role in many diseases, its role in ALI remains uncertain. This study aimed to investigate the correlation between VDBP and ALI induced by COVID-19 and further explore the potential role of VDBP in vitro studies by cellular models of acute lung injury.

In previous studies, a significant correlation between VDBP gene polymorphism and COVID-19 susceptibility was confirmed,[8–13] but there is no consistent conclusion between VDBP and the severity and mortality of COVID-19. Alabdullatif W. found that the VDBP of the critical COVID-19 was significantly higher than that of the non-critical group.[14] Povaliaeva A. also found that VDBP in COVID-19 patients was higher than in healthy people, but the correlation between VDBP and the severity was insignificant.[15] In elderly patients, ALI induced by COVID-19 brings high mortality. Whether VDBP is involved in the pathogenesis of ALI is not yet known. This study found that ALI may lead to increased serum concentration of VDBP. Elevated serum VDBP is an independent risk factor for ALI after excluding the effects of age, gender, comorbidities, mortality rate, LOS, 25(OH)D, and inflammatory indicators. Besides, the serum VDBP was positively correlated with inflammatory factors, such as WBC, CRP, SAA, PCT, and IL-6. The ROC curve showed that VDBP > 1265 ng/mL was a risk predictor for ALI in COVID-19, with a sensitivity of 84.00% and specificity of 73.85%. Although vitamin D deficiency was indicated to be associated with the incidence of acute respiratory failure of COVID-19 in previous studies,[4–6] this study found that serum VDBP levels significantly increased in COVID-19 patients with ALI and were not affected by vitamin D status. This further confirmed that VDBP has an independent biological function and may play an important role during infection or inflammation independent of vitamin D status.

Previous research discovered that VDBP exacerbates diffuse alveolar injury during the acute phase of inflammation,[19,27–29] but the exact mechanism is unclear. To explore the effect of VDBP on alveolar epithelial cells during ALI, we cultured human alveolar epithelial cells in vitro and stimulated them with LPS for inflammation. This study found that VDBP expression in alveolar epithelial cells was significantly increased after alveolar epithelial cell injury. To further understand the cause of its occurrence, we silenced the VDBP gene. In the VDBP gene silencing group, we found a significant decrease in the inflammatory factors IL-1β and TNF-α when alveolar epithelial cells were subjected to acute injury. Therefore, this study further confirms that VDBP is essential in promoting inflammatory response in ALI.

In addition, we found that in the VDBP gene silencing group, the cell viability of alveolar epithelial cells improved, and apoptosis decreased. Previous studies have found that VDBP can activate macrophages in the early stage of infection or inflammation and induce macrophage apoptosis by up-regulating cysteine protease activity via the p38 and JNK1/2 pathways.[30] However, the metabolic way of VDBP in alveolar epithelial cells is currently unknown, and further research is needed. This study confirms that VDBP accelerates apoptosis of alveolar epithelial cells in ALI. Silencing the VDBP gene has a particular protective effect on alveolar epithelial cells. This study provides more research data for understanding the pathogenesis of ALI, screening target genes, and providing new therapeutic targets.

Although many scholars emphasize the vital role of VDBP in COVID-19, this study is one of the few studies to evaluate the correlation between VDBP and ALI induced by COVID-19. Furthermore, it analyzes the effect of VDBP on alveolar epithelial cells during ALI.

Despite efforts to reduce the risk of confounding factors, this study still has many limitations. Firstly, this study is a single-center observational study. Secondly, although logistic analysis found that VDBP was an independent risk factor for COVID-19 with ALI because the OR value was only 1.001, its significance was low, and this study did not further explore the correlation between different severities of ALI and serial monitoring of serum VDBP, so more large-scale and in-depth clinical studies were still needed further to explore the value of VDBP in ALI of COVID-19. Similarly, the cutoff value of VDBP has a weaker ability to distinguish the severity of COVID-19 due to its lower sensitivity and specificity. Thirdly, due to the limitations of experimental conditions, the cell models of ALI in this study were constructed through LPS, not directly caused by SARS-CoV-2 infection.

In conclusion, this study discovered that the serum concentration of VDBP was significantly increased in COVID-19 patients with ALI, and elevated VDBP was an independent risk factor for ALI. When alveolar epithelial cells are subjected to acute injury, VDBP is vital in promoting bronchial epithelial cells' inflammatory response and apoptosis. In the future, further exploration is needed to assess the severity of ALI and its impact on the series monitoring of VDBP.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University (approval code: SZRJJ: No. 2021-411 and NO. SD NSFC 2021-0250, approval date: November 25, 2021). Informed consent was obtained from all subjects involved in the study.

Consent for Publication

All authors have approved the submission of this manuscript.

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https://www.researchsquare.com/article/rs-3555102/v1

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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