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Correlation between *rs7041* and *rs4588* polymorphisms in vitamin D binding protein gene and COVID-19-related severity and mortality

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Abstract

Background The vitamin D binding protein (DBP) plays a critical role in both innate and adaptive immune systems, participating in several clinical conditions, including coronavirus disease 2019 infection severity, and mortality rate. The study aimed to investigate the correlation between ***rs7041*** and ***rs4588*** polymorphisms in the DBP gene and Coronavirus Disease-2019 (COVID-19) severity and mortality, in patients of Suez Canal University Hospitals in Ismailia, Egypt.

Methods A case-control study enrolled 220 individuals; 140 COVID-19 patients and 80 healthy controls. Serum 25(OH) vitamin D levels were determined by the enzyme-linked immunosorbent assay (ELISA), and ***rs7041*** and ***rs4588*** polymorphisms of the DBP gene were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results The study found that both groups had vitamin D deficiency, which was considerably lower in the COVID-19 patients group compared to controls. Among COVID-19 patients, there was a significant difference in vitamin D levels according to the disease severity indicating that vitamin D levels can be used as predictors of COVID-19 severity. Negative significant correlations between genetic variants ***rs4588*** CA genotype and genetic variants ***rs7041*** TT genotype and COVID-19 prevalence ($p = 0.006$ and ***0.009*** respectively) were proved. No significant correlations between all the genetic variants of both ***rs4588*** and ***rs7041*** and COVID-19 severity ($p > 0.05$). Positive significant correlations between both genetic variants ***rs4588*** CA genotype and genetic variants ***rs7041*** TG genotype and COVID-19 mortality ($p = 0.029$ and ***0.031*** respectively).

Conclusion vitamin D deficiency increased the severity of COVID-19. The DBP polymorphism correlated with vitamin COVID-19 prevalence and mortality.

Keywords COVID-19, DBP, Polymorphism, *rs4588*, *rs7041*, Vitamin D binding protein

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Introduction

As a part of the globe, Egypt had the COVID-19 pandemic caused by "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)". Its first identification in Egypt was on February 14, 2020 [1]. COVID-19 symptoms vary considerably, while many infected people experience no symptoms or only moderate ones (such as fever, dyspnea, coughing, myalgia, or less frequently diarrhea), others experience severe life-threatening symptoms, primarily characterized by interstitial pneumonia, which frequently leads to acute respiratory distress syndrome (ARDS) and eventual mortality from respiratory failure or other consequences [2]. Older patients were more vulnerable to severe life-threatening symptoms, despite that, there were also instances of life-threatening infections among healthy people who have no health issues [3].

Regarding the virus pathogenesis, the hyperactive host immune response to COVID-19 leads to an exaggerated inflammatory reaction, including "interleukin (IL)-1β, IL-2, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-17, IL-18, IL-22, IL-33, granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, chemokine (C-X-C motif) ligand (CXCL)10, monocyte chemoattractant protein 1 (CCL2 or MCP-1), macrophage inflammatory protein 1A (MIP-1A), (CCL3), CX3CL1 and MIP-1B", such inflammatory reaction quickly cause symptoms like hypotension, fever, and edema, and in severe cases, they can even result in multiple organ failure and the host's death [4].

Vitamin D stimulates the innate immune system, particularly monocytes, and macrophages, while inhibiting the activation of the adaptive immune system. As an immune system modulator, vitamin D promotes cell differentiation while preventing cells proliferation, thus promoting the innate immune system's antimicrobial polypeptides (such as cathelicidin and β-defensin 2, that possess significant antiviral effects), and lowering the cytokine storm with influence on interferon γ (IFN-γ) and tumor necrosis factor α (TNFα) and regulating adaptive immunity through inhibiting T helper cell type 1 (TH1) and increasing the number of T regulatory lymphocytes (Tregs) [5], which are deficient in individuals with COVID-19 infection and play a significant role in combating exaggerated inflammatory reaction brought on by COVID-19, thereby maintaining homeostasis and self-tolerance [6].

The vitamin D-binding protein (DBP) gene, originally known as "Gc-globulin (group-specific component)", is vital in the transfer, and metabolism of total and free vitamin D metabolite levels; hence it is considered an essential component for various clinical conditions. DBP has "a single binding site" for all vitamin D metabolites with a high affinity for 25OHD and 1,25(OH)2D, resulting in a large pool of circulating vitamin D metabolite and preventing fast vitamin D shortage [7]. In humans, DBP is encoded by the Gc gene [8]. Being the most polymorphic protein known, the two most prevalent alleles are Gc1s (*rs7041* locus) and Gc2 (*rs4588* locus), which exhibit varying degrees of affinity for the vitamin D metabolites and have been linked to a variety of clinical problems [9]. The *rs7041* polymorphism is linked to hepatitis C virus infection, and COVID-19 infection severity [10, 11]. The *rs4588* polymorphism is linked to altered serum DBP and 25-hydroxyvitamin D levels and metabolic syndrome vulnerability [12].

Therefore, we hypothesized that DBP polymorphisms at *rs7041* and *rs4588* loci may play a significant role in COVID-19-related severity and mortality among patients of Suez Canal University Hospital, in Ismailia, Egypt.

Subject and methods

Study population and design

This prospective observational case-control research is being conducted at the Suez Canal University Hospital in Ismailia, Egypt, from February to October 2023, in the COVID-19 isolation department and the Clinical Pathology department. There were 140 patients with their first COVID-19 infection, without vaccination history, in age 26–85 years, 78 (55.7%) males and 62 (44.3%) females, Positive PCR for COVID-19: cases; and 80 healthy asymptomatic, fully vaccinated individuals of the hospital employees, in age 43–82 years, 54 (67.5%) males and 26 (32.5%) females, Negative PCR for COVID-19: control.

In Egypt, the first case of SARS-CoV-2 was announced on 14 March 2020; by the end of May 2022, there had been 513,944 confirmed cases of COVID-19 and 24,718 deaths. Egypt experienced five waves of COVID-19 by the end of May 2022, the last wave starting in the first week of the year and lasting for 16 weeks. By the beginning of the fifth COVID-19 wave, Omicron was the dominant coronavirus variant in Egypt. On 28 December 2023, the Egyptian Ministry of Health and Population announced that two patients were diagnosed with COVID-19 JN.1 variant infection, the two cases were mild infections and did not need hospitalization or Intensive Care Unit. BA.2.86.1 (JN.1's parent lineage) replication kinetics on primary nasal epithelial cells (hNEC). WHO reported that the Level of risk is low, as currently there are no reports of elevated disease severity associated with this variant, JN.1 in comparison with parent BA.2.86 lineage carries the additional spike mutation L455S that significantly enhances immune evasion capabilities [13]. Vaccination against COVID-19 started on 24th January 2021; by the end of May 2022, 46.8% of the Egyptian population were vaccinated with at least one dose, and 34.0% were fully vaccinated [14]. On 28 December 2023, the

Egyptian Ministry of Health and Population announced that two patients were diagnosed with COVID-19 JN.1 variant infection, the two cases were mild infections [13].

Ethical considerations

The current study was implemented in coordination with the guidelines of the Declaration of Helsinki. Ethical approval was gained from the Research Ethics Committee of the Faculty of Medicine, Suez Canal University, Egypt, #5088. Informed consent was obtained from the patients, which addressed all the steps of the study and their right to withdraw at any time.

Inclusion criteria

Adult patients, of both sexes (male & female), identified as COVID-19 positive by nasopharyngeal swab for detection of viral RNA by real-time PCR, in addition to baseline laboratory data and radiological findings were included in the current study.

Exclusion criteria

Minor patients, those undergoing hemodialysis, kidney transplantation, connective tissue disorders, neoplasia, or pregnancy were excluded from the current study.

Study procedure

All subjects were subjected to:

Full history taking, and thorough physical examination

As set out in the guidelines of the "Egyptian Ministry of Health and Population".

COVID-19 clinical diagnosis

Patients admitted to the isolation hospital were classified according to their grade of disease severity into five stages: (1): Asymptomatic cases; in the absence of any clinical symptoms (2). Mild cases; were considered when clinical symptoms were trivial without clinical or radiological manifestations of pneumonia. Both mild and asymptomatic cases were admitted to quarantine hospitals to reduce the incidence of viral transmission and dissemination (3). Moderate Cases; when patients have symptoms. The most common symptoms are fever, cough, headache, fatigue, breathing difficulties, anosmia, and ageusia. Pneumonia was confirmed with CT-chest (4): Severe Cases; defined by any of the following criteria: Respiratory rate > 30 breaths/min; Oxygen saturation < 93%; Arterial partial pressure of oxygen (PaO2)/Fraction of inspired oxygen (FiO2) < 300 mmHg or more than 50% progression in the chest radiological findings within 24 to 48 h (5): Critical cases; defined by any of the following criteria: respiratory failure that requires mechanical ventilation, the manifestation of shock, and other organ failures that require monitoring and treatment in the ICU [15].

Nasopharyngeal swab specimen for SARS-CoV-2 RT-PCR

Viral RNA was extracted from 250 to 300 µL of each nasopharyngeal swab using the "QIAMP VIRAL RNA micro kit (Qiagen, Hilden, Germany)" with internal PCR control, following the manufacturer's instructions. The extracted RNA was amplified immediately using the "Genesig Real-Time PCR Detection Kit for SARS-CoV-2", which included two primers/probes, one for SARS-CoV-2 detection and the other for internal extraction control detection for test validation. The cycle threshold value of (Ct) less than 34 was considered positive.

Chest computed tomography (CT) in COVID-19

The most typical CT features of COVID-19 pneumonia are bilateral and multifocal ground-glass opacities. Lesions classically predominate in the lungs' peripheral, posterior, and basal parts. Other signs have been reported such as the presence of fine reticulations, peribronchovascular thickening, vascular dilatations within pneumonia areas, or architectural distortion. Usually, there are no micronodules, excavations, septal lines, mediastinal lymph node enlargement, or pleural effusions. Some infected but asymptomatic patients may have slight ground-glass opacities but these are generally not extensive [16].

Routine laboratory testing

Blood sampling for the following tests:

- CBC (using Sysmex 5 differential part (Siemens AG, Erlangen, Germany).
- PT, PTT (using automated blood coagulation analyzer Sysmex CA1500 (Siemens AG, Erlangen, Germany).
- D-Dimer (Sterilab Services, smart tester d-dimer, code: RTC-9902-1, Mornington Terrace, Harrogate North Yorkshire, United Kingdom).
- Calcium, calcitonin, ALT, AST, Creatinine, LDH, Iron, Ferritin levels, Na, and K levels (using fully automated auto-analyzer Cobas c 6000 ("Roche Diagnostics, Mannheim, Germany").

The diagnosis technique led to three scenarios

- When chest CT was very suggestive of COVID-19, with positive RT-PCR testing, the patient was hospitalized in a COVID-19 isolation ward, with a confirmed diagnosis to be enrolled in the study.
- When chest CT was not suggestive of COVID-19, with negative RT-PCR testing, and obvious alternate

diagnosis (e.g. bacterial lobar pneumonia or left ventricular failure), the patient was hospitalized in a "non-COVID-19" ward; to be excluded from the study.

- When chest CT findings are indeterminate, RT-PCR testing along with clinical symptoms will be essential for referral to the most appropriate ward.

Serum 25-hydroxyvitamin D concentration analysis

Serum 25-OH-Vitamin-D was measured using ELISA immunoassay ("Orgentec, Diagnostika, GnbH, Mainz, Germany Kit") according to the manufacturer's instructions. The measurement range was between 5 and 200 ng/ml. The severe deficiency was <10 ng/ml, mild to moderate deficiency 10–24 ng/ml, and the optimal level was 25–80 ng/ml while>80ng/ml was considered to be a possible toxicity [17].

Genomic DNA isolation and genotyping (rs7041, rs4588) at the DBP gene

By commercially available spin column kits ("QIAamp® DNA Blood Mini Kit, QIAGEN, Stanford, Valencia, CA, USA"), genomic DNA was extracted from peripheral blood from both patients and controls. The extract will be kept at -20 °C until additional examination, and the concentration of DNA will be determined at 260 nm. There will be a polymerase chain reaction (PCR). The amplification of 482 bp PCR product around the two variants studied (*rs7041*, *rs4588*) at the DBP gene was accomplished using the following pair of primers: depending on linkage disequilibrium (LD), at the DBP gene will be amplified and detected using the following pair of primers [18]:

rs7041 (restriction enzymes HaeIII)

F. Primer (5'AAATAATGAGCAAATGAAAGAAGAC3').
R. Primer (5' CAATAACAGGAAAGAAATGAGTAGA3').

rs4588 (restriction enzymes StyI)

F. Primer (5'-AAATAATGAGCAAATGAAAGAAGAC-3'.
R. Primer (5'-CAATAACAGCAAAGAAATGAGTAGA-3'.

PCR reactions were performed using VeritiPro™ thermocycler with the reactions consisting of: "Ten micrograms of each primer, 12.5 µl of OnePCR™ Mix (2X) ("GeneDirex, Inc"), 6.5 microliters of nuclease-free water, and 3 microliters of genomic DNA". Every PCR experiment was conducted using UltraPure water called nuclease-free water such as DNases and RNases (negative control). The Separation of DNA Fragments was performed by ethidium bromide-stained 2% agarose gel electrophoresis and 100 bp DNA Ladder ("Thermo Scientific, GeneRuler"), where DNA products were visualized and captured on camera using a UV transilluminator ("Fisher Scientific™").

The "polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP)" method was used for genotyping each polymorphism. Each 482 bp PCR fragment will be digested using the *HaeIII* restriction enzyme ("Thermo Scientific™, 2000 U") to define the genotypes of the *rs7041* variant and the *StyI* restriction enzyme ("Thermo Scientific™, 2500 U") to define the genotypes of the *rs4588* variant according to manufacturer's instructions. Fragments were visualized by ethidium bromide-stained 2% agarose gel electrophoresis and 100 bp DNA Ladder ("Thermo Scientific, GeneRuler"), where DNA products were visualized and captured on camera using a UV transilluminator ("Fisher Scientific™").

The banding pattern of the agarose gel of *rs7041* polymorphism: *HaeIII* digestion created 1 band for the TT genotype (483 bp), 2 bands for the GG genotype (185, 298 bp), and 3 bands for the TG genotype (185, 298, 483 bp) using a 100 bp ladder [19]. The banding pattern on the agarose gel of *rs4588* polymorphism: *StyI* digestion created 1 band for the CC genotype (483 bp), 2 bands for the AA genotype (305, 178 bp), and 3 bands for the CA genotype (483, 305, 178 bp) using a 100 bp ladder [20].

Statistical analysis

Data collected will be reviewed, coded, and statistically analyzed using Statistical Package for the Social Science (SPSS) program version 28 (Inc, Chicago, Illinois, USA). There were descriptive statistics produced for every variable. Frequencies and percentages were used to summarize the categorical data, while the mean and standard deviation were utilized to determine if the continuous data satisfied the normal assumption. The "chi-square (χ^2)" test and "Fisher's exact" test were used in univariate analysis to find baseline differences in sociodemographic variables and health-related features. Additionally, the appropriate "one-way ANOVA" or "independent t-test" for continuous data was used for continuous variables. The "Mann–Whitney *U*" test was used for continuous data when the normality assumption was not met. Every statistical test was conducted with a two-sided significance threshold of 5%. Using simple and multivariate binary logistic regression models, factors related to the prevalence, severity, and mortality of COVID-19. Risk factor and outcome associations were shown as ORs

and 95% CIs, with $p<0.05$ being regarded as statistically significant.

Results

Baseline demographic and clinical characteristics

A total of 220 patients were included in the analysis (60% males, 40% females), and 55.5% of the participants aged more than 60 years old, with a mean age of 59.59±11.03 years in COVID-19 PCR-positive patients, and 61.68±9.85 years in controls. Both the groups were well matched in sex and age ($P=0.086$,$P=0.24$ respectively) (Table 1).

Significant differences were observed between the two groups for most of the laboratory values e.g. D dimer, ferritin, Lactate dehydrogenase (LDH), total leucocytic count (TLC), C reactive protein (CRP), NLR, and serum vitamin D level ($p<0.05$), only serum potassium level and platelet count did not differ significantly between the two groups ($p>0.05$). Patients in the COVID-19 PCR-positive patient group reported less in the D-dimer level, CRP, LDH, ferritin, and neutrophil-to-lymphocyte ratio (NLR) than controls ($p<0.001$) (Table 1).

COVID-19 PCR-positive patients displayed significantly lower median serum vitamin D levels when compared to controls (4.8 ng/ml (min-max 2.1–18) vs. 21 ng/ml (min-max 19–24), $p=0.001$). Our data showed that 140 (100%) of COVID-19 patients had low vitamin D levels with 14 (10%) having mild to moderate deficiency and 126 (90%) having severe deficiency of vitamin D. By contrast, 80 (100%) of the control group were mild to moderate vitamin D deficient (Table 1).

The study showed a significant difference in all the laboratory parameters (including vitamin D level) between the patients who have severe COVID-19 infection and patients who have mild to moderate infection ($p<0.05$) (Table 1).

60% of the COVID-19 PCR-positive cases have chronic diseases, Diabetes mellitus (DM) was the dominant comorbidity, occurring in 44.3% of participants, followed by hypertension (40%). The majority of the patients received anticoagulation and corticosteroids (95.7%) and (91.4%) respectively. A total of 67.1% of the patients required low and high-flow oxygen therapy. 30% of the cases were classified as severe cases, approximately 90%

Table 1 Comparison between the two studied groups according to different parameters

	Reference range	COVID-19 patients ($n=140$)	Controls ($n=80$)	Test of significance	*P*-value
Gender Male ($n=132$) Female ($n=88$)	- -	78 (55.7%) 62 (44.3%)	54 (67.5%) 26 (32.5%)	$\chi2=2.946$	0.086
Age (years) Median (Min. – Max.)	-	61 (26–85)	62.5 (43–82)	U = 5072	0.245
Age categories < 60 years ($n=98$) ≥60 years ($n=122$)	-	64(45.7%) 76(54.3%)	34(42.5%) 46(57.5%)	$\chi2=0.2$	0.6
TLC (cell/mm^3) Median (Min. – Max.)	4–11*10^3	11,200 (4400–67400)	7650 (4200–11442)	U = 8768	**< 0.001***
Lymphocytes (%) Median (Min. – Max.)	20–40%	10 (2–45.8)	32 (22–42.6)	U = 1036	**< 0.001***
NLR Median (Min. – Max.)	1–2	8.50 (1.10–47.50)	2.10 (1.30–3.50)	U = 10,140	**< 0.001***
Hb (g/dl) Median (Min. – Max.)	**M** 14–18 **F** 12–16	12.3 (7.6–20.1)	13.90 (11.9–17.1)	U = 2948	**< 0.001***
Platelets (mcL) Median (Min. – Max.)	150–400*10^3	276 (84–499)	272 (147–416)	U = 5274	0.473
D-dimer (ng/ml) Median (Min. – Max.)	500>	940 (100–10000)	156.5 (30–246)	U = 10,174	< **0.001***
Ferritin (ng/ml) Median (Min. – Max.)	**M** 24–236 **F** 24–307	270 (8–870)	73.5 (30–230)	U = 9594	**< 0.001***
CRP (mg/ L) Median (Min. – Max.)	8–10	68.15 (4.4–297)	5 (1–55)	U = 10,850	**< 0.001***
LDH (U/ L) Median (Min. – Max.)	140–280	295 (27–1304)	168 (110–242)	U = 8728	**< 0.001***
Creatinine (mg/dl) Median (Min. – Max.)	**M** 0.7–1.3 **F** 0.6–1.1	1.10 (0.57–11.70)	1.0 (0.70–1.30)	U = 6824	**0.007***
PT (seconds) Median (Min. – Max.)	11–13	13.25 (11.5–59)	12.20 (11.0–13.50)	U = 9480	**< 0.001***
INR Median (Min. – Max.)	0.8–1.1	1.10 (1.0–4.27)	1.0 (0.80–1.20)	U = 8540	**< 0.001***
AST (U/L) Median (Min. – Max.)	8–33	38.5 (10–287)	29.50 (10–45)	U = 7450.5	**< 0.001***
ALT (U/L) Median (Min. – Max.)	4–36	39 (10–342)	24.0 (11–36)	U = 8246	**< 0.001***
Na (mmol/L) Median (Min. – Max.)	136–145	136 (119–145)	139 (135–145)	U = 3068	**< 0.001***
K (mmol/L) Median (Min. – Max.)	3.6–5.2	4.05 (2.70–6.06)	4.0 (3.50–5.0)	U = 3068	**0.563**
25(OH)D (ng/mL) Median (Min. – Max.)	20–40	4.8(2.1–18)	21(19–24)	U = 9870	**< 0.001***
Vitamin D status				$\chi2=168.5$	**< 0.001***
Mild/moderate deficiency ($n=94$)	-	14(10.0%)	80(100%)		
Severe deficiency ($n=126$)	-	126(90.0%)	0(0%)		

χ2: Chi-square test, U: Mann–Whitney U test, p: p-value, *: Statistically significant at $p\leq0.05$, Total leukocytic count (TLC), neutrophil-to-lymphocyte ratio (NLR), LDH: lactate dehydrogenase, Variables presented as mean±SD or Median (minimum-maximum) or number of patients number (percent) as appropriate

of the COVID-19 patients improved, and only 10% of the infected cases died (Table 3).

DBP gene genotype frequencies and haplotypes analysis

Genotype frequencies at *rs7041* and *rs4588* were consistent with Hardy-Weinberg equilibrium. Concerning *rs4588* polymorphism, the homozygous major (CC) genotype was the most frequent (57.3%), while the homozygous minor (AA) genotype was the least one (4.5%) in overall study participants. The homozygous major (CC) genotype was the most common one in COVID-19 PCR-positive patients (64.3%) while the heterozygous (CA) genotype had the highest percentage in controls (50%). There was a significant association between the presence of the CC & CA genotypes and the COVID-19 infection ($p=0.005$ and 0.006 respectively). Concerning *rs7041* polymorphism, the heterozygous (TG) genotype was the most frequent (44.5%), while the homozygous minor (TT) genotype was the least one (23.7%) in overall study participants. The heterozygous (TG) genotype was the most common one in COVID-19 PCR-positive patients (44.3%) and in controls (45%). The presence of the homozygous minor (TT) genotype and the homozygous major (GG) genotypes was significantly associated with COVID-19 infection. ($p=0.009$ and 0.004 respectively) (Table 4).

Concerning haplotype analysis, the haplotype (1 F-1 S) was the most frequent (27.8%), while the haplotype (2 2) was the least one (3.7%) in overall study participants. The haplotype (1 F-1 S) was the most common one in COVID-19 PCR-positive patients (27.1%) while the haplotype (1 S-2) had the highest percentage in controls (30%). There was a significant association between the presence of the 1 F-1 S & 1 S-2 haplotypes and the COVID-19 infection ($p=0.009$ and 0.003 respectively) (Table 5).

The study revealed that there is no significant difference in serum vitamin D median levels with different *rs4588* genotypes (CC, AA, CA), and *rs7041* genotypes (TT, GG, TG) ($p=0.6$, *0.2 respectively)* (Table 6). There is no significant association between the different genotypes (rs4588 & rs7041) and COVID-19 severity ($p>0.05$) (Table 7).

Our results showed that 66.7% of the survived COVID-19 cases had the CC *rs4588* gene while 57.1% of non-survivors had the CA rs4588 gene while 41.3% of the survived COVID-19 cases had the TG rs7041 gene, also 71.4% of non-survivors had TG rs7041 gene. There was no significant association between COVID-19 mortality and the genetic variants except with rs7041 TG genotype ($p=0.03$) and rs4588 CA ($p=0.03$), also the serum vitamin D level did not differ significantly between the survived and non-survived groups ($p=0.09$) (Table 8).

Correlation between DBP gene polymorphic variations and COVID-19 prevalence, severity, and mortality

The study results showed a negative weak significant correlation ($r=-0.18$) between the CA genotype at *rs4588* and the TT genotype at *rs7041* and COVID-19 prevalence ($p=0.006$ and *0.009* respectively). There were no significant correlations between all the genetic variants of *rs4588* and *rs7041* and COVID-19 severity ($p>0.05$). Both the CA genotype at *rs4588* and TG genotype at *rs7041* are associated with increased mortality, but the association is weak ($r=0.185$ and *0.182* with $p=0.029$ and *0.031* respectively) (Table 9).

Univariate and multivariate logistic regression analysis of the various study parameters and covariates in relation to COVID-19 severity was performed. The univariate analysis showed that disease severity was decreased in the males versus the females ($p=0.02$), but not associated with age ($p=0.8$). A low serum level of vitamin D (less than 5.1 µg/ml), and increased serum levels of the following: NLR (>10.1), CRP (>70 mg/l), D-dimer (>930 ng/ml), ferritin (>370 ng/ml), LDH (>221 u/l) ($P<0.05$) correlated significantly with increased COVID-19 severity ($p=0.02$, $p=0.04$,$p<0.001$,$p<0.001$,$p=0.17$,$p=0.008$ respectively). Whereas, the multivariate analysis showed that the male gender did not correlate with COVID-19 severity ($p=0.5$). Serum vitamin D below 5.1 µg/ml is associated with increased COVID-19 severity nearly 3 times ($p=0.01$), also CRP level more than 70 mg/l is strongly correlated with COVID-19 severity ($p=0.009$) (Table 10).

Univariate and multivariate logistic regression analysis of the various study parameters and covariates in relation to COVID-19 mortality was performed. Univariate and multivariate regression showed that COVID-19-associated mortality increases with age ($p<0.001$, $p=0.01$ respectively) and disease severity ($p=0.025$, $p<0.03$ respectively). Whereas, low vitamin D levels, increased CRP levels, and the presence of chronic kidney disease were correlated with the increase in COVID-19-associated mortality in the univariate analysis ($p=0.045$, $p=0.04$, $p=0.004$ respectively) with no significant contribution to COVID-19-associated mortality in the multivariate analysis (Table 10).

Discussion

Globally, vitamin D deficiency represents a public health concern for all age groups [21]. Even in sunny nations like Egypt, deficiency and insufficiency in vitamin D are very common, especially in high-risk groups like adolescents and pregnant women. In recent cross-sectional studies, 94.8% of Egyptian healthy adolescents had vitamin D deficiency and 4.2% had vitamin D insufficiency with girls having far lower vitamin D levels than boys [22], whereas, 43.3% of the Egyptian healthy adults had

vitamin D deficiency and 25.6% had vitamin D insufficiency with women having far lower vitamin D levels than men [23]. In addition, vitamin D insufficiency was identified as a serious health issue that needed to be addressed in elderly nursing home residents in Egypt [24]. It is sad to say that the state of severe vitamin D deficiency among the Egyptian people has prompted scientists to call the situation "Vitamin D deficiency Crisis in Egypt", and to send an urgent appeal to the Egyptian Ministry of Health and Population to launch numerous campaigns to raise awareness of the dangers of vitamin D deficiency and how to prevent and treat it [25]. Even though Egypt has sunny weather, there are additional risk factors for hypovitaminosis D in the Egyptians, including insufficient dietary calcium and vitamin D, impaired vitamin D absorption, reduced vitamin D cutaneous production with aging, less time spent outside, and a sedentary lifestyle. In addition, skin pigmentation plays a role in the synthesis of vitamin D, as well as the cultural and religious customs that demand that the whole body, if not the majority of it, be covered. Gastrointestinal disorders, renal diseases, liver diseases, and central obesity also contribute to vitamin D deficiency [21, 26].

To identify host genetic factors associated with the course of COVID-19 infections, genome-wide association studies (GWAS), whole-exome sequencing (WES), and candidate gene studies have been performed by several consortia (COVID-19 Host Genetics Initiative [HGI], Genetics Of Mortality In Critical Care [GenOMICC], COVID human genetic effort, independent academic working groups, and commercial genomics service providers such as 23andMe and AncestryDNA [27]. Several single nucleotide polymorphisms (SNPs) and genes associated with infection susceptibility or distinct aspects of disease severity, such as hospitalization requirement, respiratory failure, or death were identified. Multiple GWASs have investigated host genetic variants in clinical phenotypes of COVID-19 severity/susceptibility [28, 29]. In recent years, several genome-wide association studies (GWASs) of serum 25OHD have been conducted on participants of European ancestry, to test the relationship between increased 25OHD levels and COVID-19 susceptibility and severity [30].

In the present study, laboratory tests related to COVID-19 diagnosis and follow-up indicated significant differences between COVID-19 patients and the control group. Furthermore, both the COVID-19 patients and the controls had vitamin D deficiency, being considerably lower in the group of COVID-19 patients compared to the age- and sex-matched control group. A total of 90% of COVID-19 patients exhibited a severe vitamin D deficiency compared to 0% in the control group (Table 1). Furthermore, among COVID-19 patients, there was a significant difference in such laboratory parameters, including vitamin D levels, according to the disease severity indicating that they can be used as predictors of COVID-19 severity (Table 1).

Many studies focused on laboratory testing for both diagnosis and prediction of COVID-19 severity. Viral detection with nucleic acid amplification test of lower respiratory tract specimens is the gold standard for diagnosis of COVID-19. The main routine tests requested for COVID-19 patients include complete blood count (CBC), assays investigating coagulation and fibrinolysis cascades (PT, PTT, and D-dimers), and inflammation-related parameters (Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Lactate Dehydrogenase (LDH), ferritin, and procalcitonin (PCT)), liver functions, kidney functions Due to the potential ability of the virus to severely impair several vital organs such as the heart, liver, and kidney sodium-potassium levels, etc. Because of the virus's ability to seriously damage several vital organs, including the liver, kidneys, and heart, it is acceptable for clinicians to assess the functional activities of these organs by looking at the biochemical parameters [31]. An essential tool in clinical practice at admission is the prediction of COVID-19 severity based on the laboratory test findings. This helps to anticipate severity, enhance prognosis, guide treatment, and reduce mortality rates [32, 33].

Significant heterogeneity was found across earlier studies evaluating the link of vitamin D with severity and outcomes in COVID-19 patients. Several studies reported that vitamin D is significantly associated with COVID-19 in terms of reducing disease severity, ICU hospitalization, mortality, and mechanical ventilation. They suggested Vitamin D potential as a beneficial medication for reducing the disease severity and serum levels of inflammatory markers upon Vitamin-D supplementation [34], especially vitamin D3 as oral supplementation which was found more efficacious in reducing the disease severity [35]. Vitamin D supplementation may improve the immune system of COVID-19 patients and lower its severity, especially in vitamin D deficient individuals [36] as was found in a randomized, double-blind, placebo-controlled study, where single high-dose oral cholecalciferol supplementation on ICU admission reduced the in-hospital mortality in vitamin D-deficient COVID-19 patients [37].

By contrast, no significant association of vitamin-D deficiency/insufficiency with COVID-19 severity, mortality, and ICU admissions was supposed by other studies. A negative connection was observed by Ille et al. between the mean levels of vitamin D in many European nations and the number of COVID-19 cases, and mortality [38]. One major concern with the included study was that the authors did not make enough multivariable adjustments to account for confounding. The study had an ecological

bias, which might be attributed to regional and temporal scale disparities between mean vitamin D levels at the country level. In addition, there was no significant association of vitamin D supplementation with COVID-19 and its clinical outcomes such as morbidity, mortality, ICU admission, and ventilation [39, 40]. The major limitations of such studies that could be taken into account were the inclusion of non-randomized studies, and heterogeneity in the study in forms of dose, duration, and populations. A recent study could not find a statistically significant difference in any of the secondary outcomes in severe COVID-19 patients admitted to the ICU and who need respiratory support after the daily supplementation of vitamin D [41]. The main limitation of that study was the non-randomized small number of patients included, being a single-center design rather than large multicenter studies that could provide conclusive evidence.

In the present study, the DBP genotype and haplotype frequencies of participants according to *rs4588* and *rs7041* SNPs were explored (Table 4, Table 5). No significant difference in serum vitamin D concentrations with different *rs4588* and *rs7041* genotypes (Table 6), which in turn showed no significant association with COVID-19 severity ($p>0.05$) (Table 7). By contrast, there was a significant association between COVID-19 mortality and the genetic variants *rs4588* CA ($p=0.03$) and *rs7041* TG genotype ($p=0.03$), also the serum vitamin D level did not differ significantly between the survived and non-survived groups ($p=0.09$) (Table 8).

The DBP is critical in the transfer and metabolism of total and free vitamin D metabolite levels, under the control of glucocorticoids, estrogen, and inflammatory cytokines but not by vitamin D itself, so it is considered an essential component for maintaining the natural balance of this vitamin in the body [7]. The DBP molecule is a 58 kDa glycoprotein synthesized in the liver, coded on the DBP gene, formerly known as the "***Gc*-globulin (group-specific component)**" gene located on chromosome 4 q11-q13 [42]. DBP has a high degree of polymorphism. It has been found that there are more than 120 polymorphism variants of the *GC* gene encoding DBP that have been proven to differ according to population. The most prevalent single nucleotide polymorphisms (SNP) are *rs7041* (c.1296T>G encoding D432E[D416E]) and *rs4588*, (c.1307 C>A encoding T436K [T420K]) variants which are located in exon 11 in domain III of the DBP gene, with distinct biochemical genotypes. Various combinations of *rs7041* and *rs4588* polymorphisms in the *GC* gene result in 3 essential isoforms (alleles) of DBP formerly identified as (*Gc*-1 F, *Gc*-1 S, and *Gc*-2) and to be identified now as (DBP-1 F, DBP-1 S, DBP-2) with different binding affinities for 25(OH)D3 (*Gc*-1 F>*Gc*-1 S>*Gc*-2). Because the *GC* gene has 2 copies, there are 6 different DBP phenotypes (or dipolotypes): (*Gc* 1f-1s, *Gc* 1f-1f, *Gc* 1f-2, *Gc* 1s-2, *Gc* 1s–1s, *Gc* 2–2) that can be identified from the genotypes. Haplotypes are determined by two missense variants in the *GC* gene (for example *rs7041* and *rs4588*) [43]. Single nucleotide polymorphisms (SNPs) are stably inherited, highly abundant, and distributed throughout the genome. These variations are associated not only with diversity within and among populations but also with individual responses to medication and susceptibility to diseases. In particular, positional cloning of genes for disease susceptibility depends on linkage disequilibrium (LD) and correlations among alleles of neighboring variations, reflecting "haplotypes" descended from a common, ancestral chromosome. It has become clear that chromosomally mapped and ordered SNPs can be grouped into "haplotype blocks" harboring a limited number of distinct haplotypes [44].

The *rs7041* and *rs4588* polymorphisms loci result in various protein isoforms and affect "protein stability, folding, flexibility, and aggregation; functional sites, reaction kinetics, and dependence on environmental parameters, such as pH, salt concentration, and temperature; protein expression and subcellular localization; and protein-small molecule, protein-protein, protein–DNA, and protein-membrane interactions" [45]. In fact, *rs4588* and *rs7041* are not only associated with vitamin D status in serum but also its metabolites in accordance with a person's susceptibility to several disorders [10, 12]. The two most prevalent DBP alleles, *rs7041* and *rs4588*, have been linked to the etiology of several clinical disorders [9], for example, chronic obstructive pulmonary disease (COPD) was linked to both allelic variants (*rs7041* and *rs4588*), and hepatitis C viral infection (HVC) to *rs7041* locus [46].

Previous studies suggested that genetic variants in DBP have been implicated in the circulating 25(OH) D concentrations, mainly by their affinity to vitamin D *(Gc1F>Gc1S>Gc2)*. Certain genotypes of the *rs7041* and *rs4588* alleles significantly affect vitamin D levels and also the response to vitamin D correction even at higher supplementation doses [12, 47]. Higher plasma levels of 25-hydroxy vitamin D (25(OH)D) were shown to be associated with subjects having the genetic variants *rs4588* AA genotype, while patients having genetic variants *rs7041* GG genotype have shown less 25(OH)D levels after same dose of vitamin D supplementation. This has substantial therapeutic significance because such genotypes make current vitamin D management methods ineffective, putting such people at a higher risk of deficiency of the vitamin and its associated illnesses [12]. In contrast, another study showed no significant effects of different DBP *rs4588* and *rs7041* genotypes on serum vitamin D concentrations, either at baseline or post-supplementation, which matches this study's finding [48].

Table 2 The laboratory variables according to COVID-19 infection severity in COVID-19 patients' group ($n = 140$)

	Severity of COVID-19		Test of significance	*P*-value
	Mild to Moderate ($n = 99$)	Severe ($n = 41$)		
Lymphocytes (%) Median (Min. – Max.)	8.0 (2.0 – 30.0)	11.0 (3.0 – 45.8)	U= 2551.5	**0.017***
NLR Median (Min. – Max.)	10.10 (2.40 – 47.50)	8.0 (1.10 – 31.67)	U= 1579.5	**0.039***
D-dimer (ng/ml) Median (Min. – Max.)	1200 (100 – 10000)	624.83 (100 – 10000)	U=1502.5	**0.016***
Ferritin (ng/ml) Median (Min. – Max.)	339.90 (46.7 – 870)	234.0 (8.0 – 813.0)	U=1382.5	**0.003***
CRP (mg/l) Median (Min. – Max.)	115.7 (35 – 200)	57.0 (4.4 – 297.0)	U=1087.5	**<0.001***
LDH (u/l) Median (Min. – Max.)	327 (131 – 765)	267 (27 – 1304)	U=1550.5	**0.028***
Serum Vit. D level Median (Min. – Max.)	4.40 (31 – 9.1)	5.1 (2.1 – 13.0)	U=2570.0	**0.013***

U: Mann–Whitney U test: p-value, *: Statistically significant at $p \leq 0.05$, neutrophil-to-lymphocyte ratio (NLR), LDH: lactate dehydrogenase, Variables presented as mean ± SD or Median (minimum-maximum)

The present study results showed a negative significant correlation between genetic variants *rs4588* CA genotype and genetic variants *rs7041* TT genotype and COVID-19 prevalence ($p=0.006$ and 0.009 respectively). There were no significant correlations between all the genetic variants of both *rs4588* and *rs7041* and COVID-19 severity ($p>0.05$). A positive significant correlation between both genetic variants *rs4588* CA genotype and genetic variants *rs7041* TG genotype and COVID-19 mortality ($p=0.029$ and 0.031 respectively) (Table 2).

Investigating the association between DBP gene polymorphisms and COVID-19 infection prevalence, severity, and mortalities; Speeckaert et al. discovered a negative link between DBP polymorphism and the prevalence, severity, and mortality of COVID-19 [49]. Batur et al. study found positive significant correlations between genetic variants *rs7041* TG genotype and the COVID-19 prevalence and mortality rates, while there was a negative significant correlation between the genetic variants *rs7041* TT genotype and the COVID-19 prevalence and mortality rates. No significant correlation was found at any of the variants *rs4588* locus genotypes in a variety of populations, including those in China, Japan, Nigeria, Kenya, Germany, Mexico, Italy, the Czech Republic, and Turkey [11].

In order to improve health outcomes, our study tried to identify and validate factors that may predict COVID-19 disease severity and prognosis. The study tested several clinical, laboratory, and medical predictors of COVID-19

Table 3 Distribution of the health-related characteristics and medication administered and severity and outcome in COVID-19 patients' group ($n = 140$)

Health-related condition	No. (%)
Chronic diseases	84(60.0%)
Diabetes mellitus	62 (44.3%)
Hypertension	56 (40.0%)
Ischemic Heart Disease	12 (8.6%)
Chronic Kidney Disease	12 (8.6%)
Chronic liver disease	14 (10.0%)
Others Complaint	
COPD	8 (5.7%)
Cardiomyopathy	4 (2.9%)
Atrial fibrillation	4 (2.9%)
Mitral valve diseases	2 (1.4%)
Psoriasis	2 (1.4%)
Rheumatoid arthritis	2 (1.4%)
High-Resolution CT chest	
< 50%	106 (75.7%)
> 50%	34 (24.3%)
Condition of Discharge (outcome)	
Improvement	126 (90.0%)
Death	14 (10.0%)
Oxygen Therapy	
No	20 (14.3%)
Low and high flow	94 (67.1%)
CPAP	20 (14.3%)
Intubation	6 (4.3%)
Medications	
Remdesivir	50 (35.7%)
Actemra	10 (7.1%)
Anticoagulants	134 (95.7%)
Corticosteroids	128 (91.4%)
Antibiotics	138(98.6%)
Severity*	
Mild	16 (11.4%)
Moderate	82 (58.6%)
Severe	42 (30.0%)

Data are expressed as the number and percentage of patients. * Severity according to WHO

Table 4 Distribution of DBP gene genotype variations between the two studied groups

	COVID-19 patients ($n = 140$)	Controls ($n = 80$)	Total ($N = 220$)	χ^2	*P*-value
Genotype (*rs4588*)					
CC	90 (64.3%)	36 (45.0%)	126(57.3%)	7.738	**0.005***
AA	6 (4.3%)	4 (5.0%)	10(4.5%)	0.060	0.807
CA	44 (31.4%)	40 (50.0%)	84(38.2%)	7.439	**0.006***
Genotype (*rs7041*)					
TT	24 (17.1%)	28 (35.0%)	52(23.7%)	6.837	**0.009***
GG	54 (38.6%)	16 (20.0%)	70(31.8%)	8.094	**0.004***
TG	62 (44.3%)	36 (45.0%)	98(44.5%)	1.378	0.240

$\chi 2$: Chi-square test, the p-value for comparing between the two studied groups. Variables presented as number of patients (percent), *: Statistically significant at $p \leq 0.05$

Table 5 Distribution of the compound genotypes for the two DBP gene variations (haplotypes analysis) between the two studied groups

Haplotypes name	Combination of rs7041 and rs4588 genotypes	COVID-19 patients (*n* = 140)	Controls (*n* = 80)	Total (*N* = 220)	χ2	*P*-value
1 F–1 F	TT CC	22 (15.7%)	8 (10%)	30 (13.6%)	0.138	0.299
1 F-1 S	TG CC	38 (27.1%)	23 (28.8%)	61(27.8%)	6.984	**0.009***
1 F-2	TT CA	14 (10%)	9 (11.2%)	23(10.4%)	0.060	0.125
1 S–1 S	GG CC	28 (20%)	12 (15%)	40(18.1%)	0.215	0.0601
1 S-2	TG CA	34 (24.3%)	24 (30%)	58(26.4%)	7.982	**0.003***
2–2	TT AA	4 (2.9%)	4 (5%)	8(3.7%)	1.237	0.487

χ2: Chi-square test, the p-value for comparing between the two studied groups. Variables presented as number of patients (percent), *: Statistically significant at $p \leq 0.05$

Table 6 Serum vitamin D Level (ng/ml) in different *rs4588* and *rs7041* genetic variants in COVID-19 patients' group (*n* = 140)

	N	Serum Vitamin D levels Median (min-max)	U test	*P*-value
Genotype (rs4588)			0.8	0.6
CC	106	6.2 (2.1–24)		
AA	10	9 (3.2–21)		
CA	24	11.5(2.1–23)		
Genotype (rs7041)			2.5	0.2
TT	22	15(2.1–22)		
GG	40	5.7(2.1–24)		
TG	88	7.9(3.1–24)		

Variables presented as median (minimum-maximum) p: p-value for comparing the different genotypes by Mann–Whitney U test

Table 7 COVID-19 severity in different *rs4588* and *rs7041* genetic variants in COVID-19 patients' group (*n* = 140)

	Severity of COVID-19		Total (*n* = 140)	χ2	*P*-value
	Mild to moderate (*n* = 41)	Severe (*n* = 99)			
Genotype (*rs4588*)					
CC	25 (60.98%)	65 (65.66%)	90(64.3%)	0.277	0.6
AA	0(0%)	6 (6.06%)	6(0.04%)	2.596	0.1
CA	16(39.02)	28 (28.28%)	44(31.4%)	1.522	0.2
Genotype (*rs7041*)					
TT	6 (14.63%)	18 (18.18%)	24(17.1%)	0.257	0.6
GG	13 (31.70%)	41 (41.41%)	54(38.6%)	1.153	0.3
TG	22 (53.65%)	40 (40.40%)	62(44.3%)	2.064	0.1

χ2: Chi-square test, the p-value for comparing the two studied groups. Data are expressed as the number and percentage of patients

Table 8 Distribution of vitamin D levels in different *rs4588* and *rs7041* genetic variants across the COVID-19 outcome on discharge in COVID-19 patients' group (*n* = 140)

	Mortality of COVID-19		χ2	*P*-value
	Survived (*n* = 126)	Non survived (*n* = 14)		
Genotype (*rs4588*)				
CC	84(66.7%)	6(42.9%)	3.1	0.07
AA	6(4.8%)	0(0.0%)	0.7	0.4
CA	36(28.6%)	8(57.1%)	4.7	**0.03***
Genotype (*rs7041*)				
TT	24(19.0%)	0(0.0%)	3.2	0.07
GG	50(39.7%)	4(28.6%)	0.6	0.4
TG	52(41.3%)	10(71.4%)	4.6	**0.03***
Vitamin D Median (min -max)	4.2(3.2–6.1)	4.8(2–13)	U = 567	0.09

χ2: Chi-square test, the p-value for comparing the two studied groups. Data are expressed as the number and percentage of patients, *: Statistically significant at $p \leq 0.05$

Table 9 The correlation between different genotypes and disease prevalence, severity, and mortality

Spearman's Rho	Genotype (rs4588)			Genotype (rs7041)		
	CC	AA	CA	TT	GG	TG
Prevalence						
r	0.19	-0.016	-0.18	-0.18	0.219	-0.079
P-value	**0.005***	0.808	**0.006***	**0.009***	**0.001***	0.242
Severity						
r	-0.033	-0.139	0.094	-0.050	-0.070	0.107
P-value	0.703	0.103	0.269	0.560	0.408	0.210
Mortality						
r	-0.149	-0.071	0.185	-0.152	-0.068	0.182
P-value	0.079	0.408	**0.029***	0.074	0.421	**0.031***

r is Spearman's rho coefficient, * statistically significant at $p \leq 0.05$

severity and outcome in infected patients. Our results indicated that D Dimer, CRP, LDH, ferritin, neutrophils to lymphocyte ratio (NLR), and vitamin D level could greatly differentiate COVID-19 patients into severe illness and mild to moderate disease. The ideal cut-off value for vitamin D3 level was found to be 5.1 µg/ml. Vitamin D was revealed to have a substantial protective effect against COVID-19 mortality ($p<0.05$) in the univariate study (Table 10).

Searching for risk factors of COVID-19 has become the main concern of all coronavirus-interested scientists. Identification of potential risk variables that predict disease progression may be extremely useful for healthcare providers in efficiently triaging patients, personalizing treatment, monitoring clinical progress, and allocating appropriate resources to reduce morbidity and death [50]. In fact, the determinants of COVID-19 disease severity and mortality risk are not well studied till now, but several emerging studies highlighted a wide range of patient variables, including demographics (e.g. old age), pre-existing chronic comorbidities (e.g. cardiovascular disease, chronic kidney disease, chronic lung diseases (particularly COPD), diabetes mellitus, hypertension, immunosuppression, obesity, etc.), extensive lung involvement in form of hypoxia and/or specific chest CT findings, and certain laboratory test results deterioration (e.g. coagulation profile abnormalities, cardiac biomarkers dysfunction, alterations in white blood cell (WBC) counts, altered liver functions, altered kidney functions, non-specific biomarkers of cellular injury as LDL, etc.) [50–53].

Just now we can say with confidence that vitamin D possesses a promising role as a predictor of COVID-19 severity and outcomes of hospitalized patients, depending on vitamin D deficiency correlation with increased mortality and a greater incidence of severe COVID-19 [54–57]. A pilot clinical trial recently demonstrated that administering a high dose of vitamin D3 significantly reduced the need for ICU treatment of COVID-19 patients [58], and a second report claimed that high-dose vitamin D therapy can shorten the length of stay for COVID-19 patients [59].

It is worth mentioning that vitamin D deficiency impacts on COVID-19 clinical outcomes remains controversial. A meta-analysis of eleven cohort studies with "536,105 patients" and "two randomized clinical trials" claimed that "Vitamin D deficiency (<20 ng/ml)" or "vitamin D insufficiency (<30 ng/ml)" showed no correlation with increased risk of COVID-19 infection or in-hospital death [60]. Despite such findings, low vitamin D levels in corporations with many other factors, such as age, comorbidities, and disease severity, can be used as predictors for negative outcomes such as respiratory support needs and mortality [57].

In order to understand why DBP was linked to COVID-19 severity, prevalence, and mortality rates, we must know that the majority (85–90%) of 25-hydroxyvitamin D in the circulation is bound to DBP, whereas 10–15% is loosely bound to albumin, and <1% circulates in its free form. Besides the transport of vitamin D and its metabolites, DBP could exert several other key roles in COVID-19, being involved in the immunomodulatory effect of bioavailable vitamin D levels of patients, which is determined by the genetic background in addition to public awareness, behaviors, and antiviral policy of each country. On the contrary, the severity of the disease might be associated with the genetic host factors only [61]. DBP is also involved in "the extracellular actin-scavenger system" acting as a "neutrophil chemotactic factor" and a "macrophage activator" [62]. The interplay between vitamin D and angiotensin-converting enzyme 2 (ACE2) is also an influential factor in the correlation between vitamin D and its binding protein and COVID-19-related outcomes. COVID-19 virus enters human cells by ACE2, a component of the renin-angiotensin system (RAS), and reduces its expression, leading to lung damage and pneumonia. Vitamin D is a negative endocrine RAS modulator that decreases renin expression and production. It activates the "ACE2/Ang-(1-7)/MasR axis" while inhibiting "renin and the ACE/Ang II/AT1R axis". This increases

Table 10 Univariate and multivariate Logistic regression analysis for the parameters affecting COVID-19 severity and COVID-19 associated mortality (*N* = 140)

	COVID-19 severity				**COVID-19 mortality**			
	Univariate		**#Multivariate**		**Univariate**		**#Multivariate**	
	OR (95% C. I)	***P* value**	**OR (95% C. I)**	***P* value**	**OR (95% C. I)**	***P* value**	**OR (95% C. I)**	***P* value**
Male	0.39 (0.17–0.86)	**0.02***	0.73 (0.25–2.17)	0.5	0.5(0.18–1.67)	0.3		
Age	1.01 (0.97–1.04)	0.8			1.17)1.08–1.28)	**< 0.001***	1.18 (1.04–1.34)	**0.01***
Low vitamin D	2.61 (1.16–6.18)	**0.023***	3.61 (1.37–10.4)	**0.01***	0.64 (0.41–0.99)	**0.045***		
High NLR	2.25 (1.01–5.08)	**0.041***	1.19 (0.38–3.59)	0.8	1.05 (0.99–1.11)	0.08		
High CRP	7.69 (3.06–22.3)	**< 0.001***	5.80 (1.60–22.8)	**0.009***	1.01 (1.0-1.02)	**0.04***		
High D-dimer	4.51 (1.98–10.7)	**< 0.001***	1.05 (0.27–4.12)	> 0.9	1 (1-1.01)	0.7		
High ferritin	2.75 (1.20–6.33)	**0.017***	1.59 (0.54–4.71)	0.3	1 (1.00-1.1)	0.6		
High LDH	4.55 (1.62–16.3)	**0.008***	2.98 (0.83–13.2)	0.12	1 (0.99- 1)	0.3		
Genotype (*rs4588*)								
CC (reference)								
AA	0.00 (00–00)	>0.9			0.00 (00–00)	0.9		
CA	1.08(0.44–2.54)	>0.9			1.08(0.44–2.54)	0.9		
Genotype *(rs7041)*								
TT (reference)								
GG	1.65(0.50–6.5)	0.4			1.65(0.50–6.52)	0.4		
TG	1.60(0.49–6.2)	0.5			1.60(0.49–6.24)	0.5		
Severity					4.61 (1.23–19.1)	**0.025***	5.78(1.12–29.7)	**0.03***
Diabetes					1.78 (0.58–5.42)	0.3		
Hypertension					2.2 (0.71–6.62)	0.175		
IHD					0	0.9		
CKD					8 (1.93–33.1)	**0.004***	0.827(0.08–8.4)	0.8
Chronic liver D					1.5 (0.32–7.93)	0.57		
Medications								
Corticosteroids					0.34(0.07–2.49)	0.21		
Remdesivir					0.28(0.06–1.32)	0.12		
Actemra					0	> 0.9		
Anticoagulant					NA	0.9		

OR: Odd's ratio, C.I: Confidence interval, IHD: Ischemic Heart Disease, CKD: Chronic kidney Disease

#: All variables with $p < 0.05$ in the univariate analysis were included in the multivariate, *: Statistically significant at $p \leq 0.05$, Low-level vitamin D: serum vitamin D level less than 5.1, High NLR: NLR ratio > 10.1, High CRP: CRP serum level > 70, High D dimer: serum D-dimer level > 930, High ferritin: serum ferritin level > 370, High LDH: serum LDH level > 221

the expression and concentration of ACE2, MasR, and Ang-(1-7), potentially protecting against acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) [63]. As an immune modulatory, vitamin D promotes the innate immune system's antimicrobial polypeptides production by monocytes and macrophages and lowers the cytokine storm by inhibiting T helper cell type 1 (TH1) and increasing the number of T regulatory lymphocytes (Tregs) [6].

Our study had a few limitations. First, this study had a small sample size and was conducted at a single center in Suez Canal University Hospitals in Ismailia, Egypt. Second, long-term follow-up was not done because of the short time for data collection.

Conclusion

Lower serum concentrations of vitamin D may potentially make certain patients more prone to a more severe course of COVID-19.

The DBP polymorphism may have an impact on the connection between vitamin D and COVID-19 prevalence and mortality.

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Author contributions

E.R. Hamed: concept and design of the study, data acquisition, statistical analysis, interpreted the results, analyzed the data, drafted the manuscript, critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work. S.A. Abdelhady: concept and design of the study, data acquisition, analyzed the data, drafted the manuscript, critically revised the manuscript, and approved the final version to be published. S.A. Al-Touny: concept and design of the study, data acquisition, analyzed the data, drafted the manuscript, critically revised

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Data availability
The dataset generated and analyzed during our study is available in the "Genbank with accession #OP032652.1", https://www.ncbi.nlm.nih.gov/nuccore/OP032652.1.

Declarations

Human ethics and consent to participate
Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Suez Canal University, Egypt, #5088. All subjects were informed and gave their voluntary, written informed consent. It is declared that: All methods were carried out in accordance with relevant guidelines and regulations. It was performed according to the recommendations of Good Clinical Practice and the Declaration of Helsinki (2013).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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