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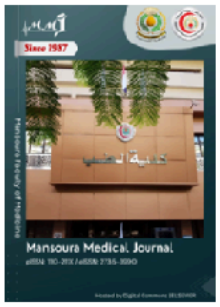

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Association of vitamin D receptor Cdx-2 polymorphism with COVID-19: A case-control study

Mahmoud M. Alseoudy
Department of Anesthesia and surgical intensive care, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt, drs3ody.mansora@mans.edu.eg
Maha O. Hammad
Department of Medical Biochemistry and molecular biology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Mohmed Elgamal
Chest Medicine Department, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Dalia A. Abdelghany
Chest Medicine Department, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Asmaa M. Borg
Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

See next page for additional authors

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Mahmoud M. Alseoudy, Maha O. Hammad, Mohmed Elgamal, Dalia A. Abdelghany, Asmaa M. Borg, Ahmed El-Mesery, and Dina Elzeiny

ORIGINAL STUDY

Association of Vitamin D Receptor Cdx-2 Polymorphism With COVID-19: A Case–Control Study

Mahmoud M. Alseoudy [a,*], Maha O. Hammad [b], Mohmed Elgamal [c], Dalia A. Abdelghany [c], Asmaa M. Borg [d], Ahmed El-Mesery [e], Dina Elzeiny [b]

[a] Department of Anesthesia and Surgical Intensive Care, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[b] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[c] Department of Chest Medicine, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[d] Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[e] Department of Tropical Medicine, Mansoura University Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Abstract

Background: **Host genetic variability has been suggested as an important explanation for inter-individual differences in COVID-19 susceptibility and severity. Most vitamin D actions in the regulation of immunity are mediated by vitamin D receptors (VDRs). Polymorphisms in the *VDR* gene have been associated with several health outcomes; however, their effects on COVID-19 still need more clarification. This study aims to investigate the association of the VDR SNP (rs11568820, Cdx-2) with susceptibility and interindividual variability of the severity of COVID-19.**

Methods: **A total of 100 confirmed COVID-19 patients and 100 age and sex-matched controls were enrolled in this study between July and September 2021. COVID-19 patients were further subdivided into severe ($n = 50$) and nonsevere ($n = 50$) cases. All participants were subjected to genotyping of Cdx-2 SNP using the allelic discrimination of the Real-time PCR technique and assay of serum 25(OH)D levels by ELISA.**

Results: **The results showed that the homozygous "GG" genotype was significantly higher in patients vs. controls, whereas the heterozygous "AG" genotype was significantly lower in COVID-19 patients. Thus, the heterozygous "AG" genotype is considered the protective genotype. This protection was more significant among males vs. females ($P = 0.02$). However, there were no statistically significant differences in the genotype distributions of VDR Cdx-2 SNP between severe and nonsevere patients. Moreover, COVID-19 patients with the "AG" genotype presented higher 25(OH)D levels than the "GG" genotype ($P = 0.02$).**

Conclusions: **VDR SNP (rs11568820, Cdx-2) might be a potential risk factor for COVID-19, particularly among male patients.**

Keywords: COVID-19, Real-time PCR, VDR Cdx-2 polymorphism, Vitamin D

1. Introduction

Coronavirus disease 2019 (COVID-19) is a viral pneumonia with different levels of severity from the complete absence of symptoms up to cytokine storm and serious acute respiratory distress syndrome (ARDS) (Guan et al., 2020; Conti et al., 2020; Pascarella et al., 2020). Vitamin D is an essential regulator of natural and acquired immunity (Caprio et al., 2017; Fabbri et al., 2020) with antiviral properties through immune-modulation, anti-inflammatory impacts, and direct interference with the replication of viruses, which can provide beneficial effects to SARS-CoV-2 infection (Teymoori-Rad et al., 2019; Guo et al., 2020).

Low serum vitamin D levels are commonly found in the elderly or those with chronic conditions, such as diabetes, hypertension, or cancer, and have also

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* Corresponding author at: Abdelsalam Aref St., Mansoura City, El-Dakahliya Governorate, Mansoura, 3551, Egypt. Te.: +201006224551.
E-mail address: drs3ody.mansora@mans.edu.eg (M.M. Alseoudy).

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been reported as poor prognostics for COVID-19 (Holick, 2017; Infante et al., 2019; Zhou et al., 2020). Most vitamin D actions are mediated by vitamin D receptors (VDRs). The VDR has been recognized in neutrophils and antigen-presenting cells (White, 2012), as well as in Type-II pneumocytes (Zdrenghea et al., 2017) that are considered the primary target of coronaviruses (Ebadi and Montano-Loza, 2020).

VDR single-nucleotide polymorphisms (SNPs) are associated with augmented susceptibility or resistance to respiratory infections (Jolliffe et al., 2018). The VDR SNP (rs11568820, Cdx2) is located in the promoter region of the *VDR* gene and comprises a binding site for the transcription factor CDX2 (caudal type homeobox-2) in which the major wild allele G exhibits less transcriptional activity compared to minor mutant allele A (Ntais et al., 2003). The VDR SNP (rs11568820, Cdx2) was linked with susceptibility and severity of diseases accompanied by an imbalance in immunity (Meyer and Bornman, 2018).

The potential role of vitamin D-related gene polymorphisms in the prevention and treatment of COVID-19 is currently the topic of several studies; however, few studies have investigated the possible association between the VDR SNP (rs11568820, Cdx-2) and COVID-19. Thus, this study aims to investigate this linkage in Egyptians. Our findings may help identify the susceptibility of COVID-19 and may explain the inter-individual variability of the severity of COVID-19 patients.

2. Subjects and methods

2.1. Study population

This case–control study was performed in the Medical Biochemistry and Molecular Biology Department, Mansoura Research Centre for Cord Blood Stem Cells (MARC), Clinical Pathology Department, and Mansoura University Hospitals, Faculty of medicine, Mansoura University between July and September 2021. This research was consistent with the Declaration of Helsinki and accepted by the Institutional Research Board (IRB) (approval code: R.20.06.901). Written informed consents were obtained from all study contributors.

The present study included 200 subjects divided into two groups. The control group comprised 100 healthy participants with a negative rapid test. The other COVID-19 patient group included 100 cases were diagnosed the quarantine department of Mansoura University hospitals with PCR-positive results for SARS-CoV-2. COVID-19 patients were further subdivided into a severe group ($n = 50$) included severe to critically ill cases, and the non-severe group ($n = 50$) included mild-to-moderate cases.

Severity assessment was according to criteria of Ministry of Health and Population (MOHP), Egypt: Management protocol for Covid-19 patients November 2020 in which nonsevere cases included both mild and moderate COVID-19 in which mild cases are cases who have mild symptoms and normal non-contrast computed tomography (CT), whereas moderate cases show CT features of COVID-19 and peripheral capillary oxygen saturation (SpO_2)≥92%. Severe cases displayed any of the following: respiratory rate>30 breaths/min, SpO_2<92% at room air, PaO_2 (arterial oxygen partial pressure)/FiO_2 (fraction of inspired oxygen) < 300, chest radiology showing>50% lesion. The critically ill cases showed respiratory failure, septic shock, and/or multi-organ dysfunction.

The following subjects were excluded from the study: Subjects who had any vitamin D supplements for the last 2 years, patients with any health problem that would affect vitamin D serum levels, non-confirmed COVID-19 patients, children and pregnant COVID-19 patients. Data about sex, age, previous treatment, and comorbidities were collected for all study contributors. For COVID-19 cases, complete blood count, international normalized ratio, D-dimer, lactate dehydrogenase, C-reactive protein, and CT chest were performed. The clinical outcome (either improved or dead) and the need of mechanical ventilation were obtained.

2.2. Samples collection

Five millilitres of peripheral blood were received from all study participants. Each sample was divided into two different tubes: Two ml of blood were added to an ethylene di-amine tetra acetic acid tube for DNA extraction, and the other 3 ml were added to a dry tube and centrifuged for 15 min at 3000 rpm to obtain serum for vitamin 25(OH)D assessment.

2.3. Genotyping of the VDR SNP (rs11568820,Cdx2) using the allelic discrimination real-time PCR technique

Genomic DNA was isolated using a QIAamp Blood Mini Kit **(Qiagen, Hilden, Germany)** according to the manufacturer's instructions, and its quantity was assessed using a Nanodrop 2000 UV Visible spectrophotometer **(Thermofisher Scientific, Waltham, USA)**, and then kept at −20 °C until analysis.

The TaqMan real-time PCR mixture was prepared as follows: 10 ng template DNA, 1.25 μl of 20x SNP Genotyping Assay *(Applied Biosystems, Foster City, USA)*, 12.5 μl of 2x genotyping Master Mix, and complete with nuclease-free water to 25 μl as a total volume. Reaction setup was as follows: initial denaturation for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The genotype analysis was performed using 7500 Real-Time PCR apparatus according to the relative fluorescent signal strength of VIC and FAM dyes (*Applied Biosystems, Foster City, USA).*

The 20x SNP Genotyping Assay contained probes and primers. Two TaqMan allele-specific probes, only differ in sequence at the Cdx-2 G < A (rs11568820) SNP site, were used in genotype analysis of extracted DNA. One probe was completely base paired with the G (wild) allele and the other one base paired with the A (mutant) allele. Each probe had a reporter fluorescent VIC or FAM dye as a label. The Cdx2 (rs11568820) probe sequence: ACCCATAA-TAAAGAATAAGTTTTTA [C/T]TGTAGCC-TAGTTTACTCAGAGATAT; the C probe detected the G allele (wild-type) while the T probe detected the A allele (variant-type). The result analysis was according to the dye released in each sample. Release of the VIC green dye indicated a homozygous wild GG genotype, whereas the release of the FAM blue dye referred to the homozygous mutant AA genotype. When both dyes are released, it means heterozygosity for both alleles (Fig. 1).

2.4. Assay of serum 25(OH)D levels by ELISA

Vitamin D levels were estimated in the sera of subjects using a 25-Hydroxy vitamin D competitive binding ELISA kit **(Calbiotech, El Cajon, USA)** according to the manufacturer's instructions. Anti-Vitamin D antibody coated wells were incubated with Vitamin D samples, standards, and vitamin D-Biotin conjugate. A fixed amount of biotin-labeled vitamin D competed with the endogenous Vitamin D in the standards and samples for a limited of binding sites on the anti-Vitamin D antibody. After washing, Streptavidin-Horseradish Peroxidase (SA-HRP) was used to detect bound Vitamin D-Biotin. The amount of SA-HRP conjugate bound to the well was inversely proportional to serum Vitamin D level. Then, Unbound SA-HRP conjugate was washed and tetramethylbenzidine reagent was added and incubated resulting in a blue color.

An acidic stop solution was then added to stop the color reaction, and the absorbance was measured via Infinite F50 ELISA Reader at 450 nm spectrophotometrically **(TECAN, Männedorf, Switzerland).** By plotting the standards concentrations versus the absorbances, a standard curve was established. The intensity of the color was inversely proportional to 25-OH Vitamin D concentration in the sample.

2.5. Statistical analysis

SPSS **(version 25.0, IBM, Chicago, IL, USA)** and SNPStats software were used to analyze and interpretate data. A chi-square test was utilized for the comparison of qualitative data. Kolmogorov–Smirnov test was utilized to test for normality of quantitative data. Non-normally distributed data were expressed as median and 25th percentile–75th percentile and compared using the Mann–Whitney U-test. The VDR SNP (rs11568820, Cdx-2) was tested for Hardy–Weinberg equilibrium then the association between the genotypic and allelic frequency and Covid-19 vulnerability and severity was performed. The inheritance models (co-dominant, dominant, recessive and over-dominant) were checked to identify the best inheritance model with the lowest P, Akaiki information criterion, and Bayesian information criterion values. For all used tests, results were statistically significant if P value was$\leq$0.5.

3. Results

3.1. Demographic criteria and 25(OH)D levels of COVID-19 patients and controls

Table 1 shows the demographic characteristics of subjects. COVID-19 patients included 56 males and 44 females with a median age of 54.5 years. In the controls, there were 60 males and 40 females with a median age of 48 years. The age and sex of controls were frequently matched with cases. Serum 25(OH) D level was statistically significantly lower in COVID-19 patients vs. controls (median of 29.8 vs. 43 ng/ml, $P = 0.006$).

3.2. COVID-19 patients' characteristics

As presented in Table 2, severe cases were significantly older than nonsevere cases (median: 61 vs. 40 years; $P < 0.001$). In the case of single comorbidity, there was no significant difference between severe and nonsevere cases, but severe cases tended to have more than one comorbidity than nonsevere cases. Severe cases were more prone to die and to progress to invasive mechanical ventilation ($P < 0.001$) than nonsevere cases. Regarding laboratory data, severe patients had a significant decrease in lymphocytes ($P = 0.04$) and platelets ($P < 0.001$). There was a significant increase in D-

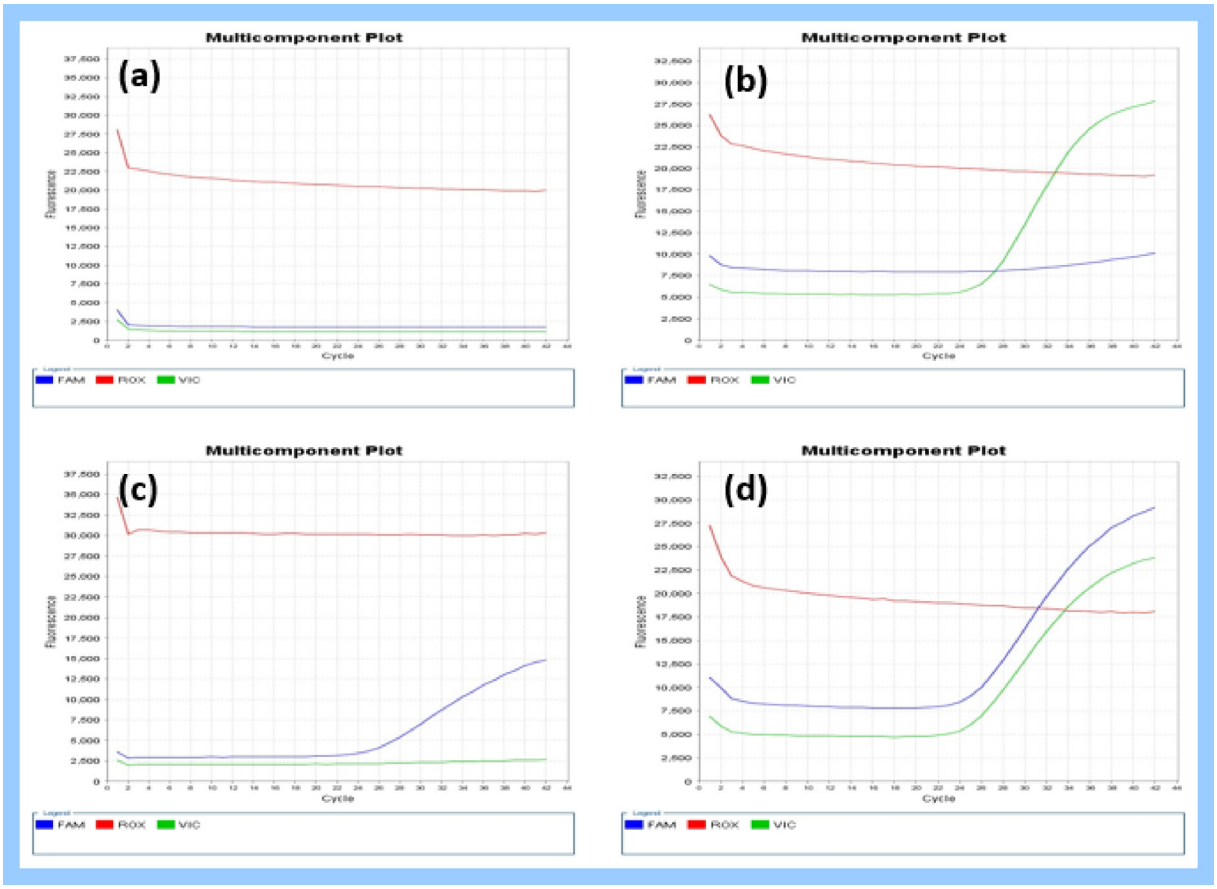

Fig. 1. Real-time PCR genotyping results of the VDR SNP (rs11568820, Cdx-2): (a) negative control; (b) homozygous GG (wild type) genotype; (c) homozygous AA (variant) genotype; (d) heterozygous AG genotype.

dimer ($P = 0.01$) and lactate dehydrogenase ($Pv = 0.001$) in severe than nonsevere patients.

3.3. Allelic and genotypic frequencies of VDR SNP (rs11568820, Cdx-2)

Allele and genotype frequencies of VDR SNP (rs11568820, Cdx-2) in COVID-19 patients and controls, as well as in severe and nonsevere COVID-19 patients are shown in Table 3. Genotype distributions were consistent with Hardy–Weinberg equilibrium for the Cdx-2 SNP (as evidenced by P value of 0.66 and 0.53 in the control and the nonsevere COVID-19 groups, respectively).

The homozygous 'GG' genotype was found to be statistically significantly higher in COVID-19

Table 1. Demographic characteristics and 25(OH) D levels of COVID-19 patients and controls.

Parameter	COVID-19 patients ($n = 100$)	Healthy controls ($n = 100$)	Statistic	P value
Age (years)	54.5 (37.3–63)	48 (44–65)	$Z = -0.851$	0.39
Sex			$\chi^2 = 0.328$	0.56
Male	56	60		
Female	44	40		
Serum 25(OH) D levels (ng/mL)	29.8 (18.8–47.2)	43 (20.3–66)	$Z = -2.761$	**0.006**
Vitamin D category			$\chi^2 = 3.371$	0.18
Deficiency (<20 ng/mL)	28a	24a		
Insufficiency (20–<30 ng/ml)	22a	14a		
Normal (≥30 ng/ml)	50a	62a		

For age and vitamin D levels, data are expressed as median (25th percentile – 75th percentile) and compared by Mann–Whitney *U*-test. For sex and vitamin D category, data are expressed as count and percentage and compared by Chi–Square test. Z-test for column proportions (with adjusted P value by Bonferroni method) is presented by letters; similar letters = insignificant difference while different letters = significant difference.

Table 2. Patients' characteristics of COVID-19 cases.

Parameter	Total COVID-19 patients ($n = 100$)	Severe cases ($n = 50$)	Nonsevere cases ($n = 50$)	statistic	P
Demographic characteristics					
Age	54.5 (37.3–63)	61 (53.8–66.3)	40 (32–55.3)	$Z = -5.086$	[a]<0.001
Sex					
Male	56	29 (58%)	27 (54%)	$\chi^2 = 0.162$	[b]0.68
Female	44	21 (42%)	23 (46%)		
Presence of comorbidities					
No comorbidities	42	14 b	28 a	$\chi^2 = 8.693$	[b]0.01
Presence of one comorbidity	25	14 a	11 a		
Presence of > one comorbidity	33	22 b	11 a		
Laboratory characteristics					
Hemoglobin (g/dl)	12.4 (10.8–13.4)	12.4 (10.0–13.2)	12.4 (11.0–13.5)	$Z = -0.925$	[a]0.35
WBCs ($\times 10^3/mm^3$)	7.6 (5.9–11.8)	8.4 (5.8–13)	7.2 (6.0–9.0)	$Z = -1.563$	[a]0.11
Lymphocytes ($\times 10^3/mm^3$)	1.4 (0.9–1.9)	1.3 (0.9–1.7)	1.5 (1.3–2.0)	$Z = -2.041$	[a]0.04
Platelets ($\times 10^3/mm^3$)	203 (148–252)	170 (138–226)	232 (195–280)	$Z = -3.641$	[a]<0.001
INR	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.0 (1.0–1.1)	$Z = -1.671$	[a]0.09
D-dimer (ng/mL)	250 (170–460)	315 (200–735)	190 (129–390)	$Z = -2.456$	[a]0.01
CRP (mg/L)	26.8 (10.5–58.5)	32.5 (12.0–68.3)	24 (9.5–47.8)	$Z = -1.283$	[a]0.19
LDH (U/L)	701 (361–989)	793 (647–1133)	340 (250–668)	$Z = -3.164$	[a]0.001
Clinical outcome					
Invasive mechanical ventilation				$\chi^2 = 19.048$	[b]<0.001
Not required	70	25 (50%)	45 (90%)		
Required	30	25 (50%)	5 (10%)		
Outcome				$\chi^2 = 17.334$	[b]<0.001
Improved	71	26 (52%)	45 (90%)		
Dead	29	24 (48%)	5 (10%)		

Abbreviations: CRP, C-reactive protein; INR, International normalized ratio; LDH, Lactate dehydrogenase; WBCs, White blood cells.
[a] Data expressed as median (25th percentiles – 75th percentiles) and compared by Mann–Whitney *U*-test.
[b] Data are expressed as count and percentage and compared by Chi–Square test. Z-test for column proportions (with adjusted *P* value by Bonferroni method) is presented by letters; similar letters = insignificant difference while different letters = significant difference.

patients versus. Controls, while the heterozygous "AG" genotype was statistically significantly higher in controls ($P <$ v0.05). Thus, the heterozygous "AG" genotype is considered the protective genotype. However, there were no statistically significant differences in the genotype distributions of VDR Cdx-2 SNP between severe and nonsevere patients. Regarding the allele distribution, there was no statistically significant difference in the distribution of "G" vs. "A" allele between cases and controls ($P = 0.12$), as well as, in severe and nonsevere COVID-19 patients ($P = 0.11$).

When the association of different inheritance models for Cdx-2 SNP and the risk of COVID-19 vulnerability was investigated, the over-dominant model showed the best inheritance model, in which the participants with 'AG' heterozygous genotype had 0.5 times lower odds (lower probability) to exhibit COVID-19 (protective genotype) compared with participants with either of the two homozygote genotypes ("AA"+"GG") [$P = 0.01$, 95% CI = 0.28–0.9] (Table 4). This protection was more significant among male vs. female participants ($P = 0.02$) (Table 5).

Table 3. Allele and genotype distribution of VDR SNP (rs11568820, Cdx-2) in COVID-19 patients and healthy controls, as well as, in severe and non-severe COVID-19 patients.

	COVID-19 patients ($n = 100$)	Healthy controls ($n = 100$)	P	Severe COVID-19 patients ($n = 50$)	Non-severe COVID-19 patients ($n = 50$)	P
Genotype						
'GG'	57 a	42 b		33 a (66%)	24 a (48%)	
'AG'	32 a	48 b	0.043	12 a (24%)	20 a (40%)	0.17
'AA'	11 a	10 a		5 a (10%)	6 a (11%)	
Allele						
'A' allele	54 (27%)	68 (34%)	0.128	22	32	0.11
'G' allele	146 (73%)	132 (66%)		78	68	

Data are expressed as count and percentage and compared via Chi–Square test. Z-test for column proportions (with adjusted *P* value by Bonferroni method) is presented by letters; similar letters = insignificant difference while different letters = significant difference.

Table 4. Inheritance model analysis of the VDR SNP (rs11568820 in association with the risk for COVID-19 (n = 200, adjusted for age and Sex).

Model	Genotype	Control	Case	OR 95%CI	P	AIC	BIC
Co-dominant	'GG'	42	57	Reference	0.06	280	296.5
	'AG'	48	32	0.49 (0.27–0.89)			
	'AA'	10	11	0.86 (0.33–2.23)			
Dominant	'GG'	42	57	Reference	0.03	279.3	292.4
	'AG- AA'	58	43	0.55 (0.31–0.97)			
Recessive	'GG-AG'	90	89	Reference	0.73	283.5	296.7
	'AA'	10	11	1.18 (0.47–2.94)			
Over dominant	'GG-AA'	52	68	Reference	0.01	278.1	291.3
	'AG'	48	32	0.5 (0.28–0.9)			

Abbreviations: 95%CI, 95% confidence interval; AIC, Akaiki information criterion; BIC, Bayesian information criterion; OR, Odds ratio.

3.4. Association between the VDR SNP (rs11568820, Cdx-2) and 25(OH)D levels among COVID-19 patients

COVID-19 patients with "AG" and "AA" genotypes presented with significantly increased 25(OH)D levels compared with patients with "GG" genotype ($P = 0.02$). The 'AG' patients exhibited the highest vitamin D levels (median: 32.84, 25th–75th percentile: 24.4–50.9 ng/ml), compared with "GG" patients (median: 22.4, 25th–75th percentile: 17.4–31.5 ng/ml) and "AA" patients (median: 24.7, 25th–75th percentile: 14.23–38.3 ng/ml) (Fig. 2).

4. Discussion

Host genetic variability has been suggested as an important explanation for inter-individual differences in COVID-19. Recognizing the role of the genetic determinants in the heterogeneity of COVID-19 vulnerability may help provide answers to disease pathogenesis, and guide the identification of the high-risk patients for early therapeutic interference and vaccination (Pereira et al., 2020).

Vitamin D is implicated in multiple immune–regulatory processes and has shown benefits in certain viral respiratory infections, including SARS-CoV-2 (Mitchell, 2020). Vitamin D can influence the transcription of many genes involved in immune-regulated processes by binding to its receptor, VDR. Allelic variations in the *VDR* gene can lead to notable receptor dysfunction, which would compromise the vitamin D action and affect the immune response. Moreover, SNPs in the regulatory regions of *VDR* can affect its expression (de Albuquerque Borborema et al., 2020).

Table 5. VDR SNP and Sex cross-classification interaction (n = 200, adjusted for age).

	SNP	Control	Case	OR (95% CI)	*P* value
Female	'GG'	26	25	1.0 (Reference)	
	'AG'	24	14	0.60 (0.25–1.41)	
	'AA'	10	5	0.53 (0.16–1.79)	0.02
Male	'GG'	16	32	1.0 (Reference)	
	'AG'	24	18	0.38 (0.16–0.90)	
	'AA'	0	6	–	

Abbreviations: OR, Odds ratio; 95%CI: 95% confidence interval; SNP, single nucleotide polymorphism.

The VDR SNP (rs11568820, Cdx-2) is one of the regulatory region SNPs with the ability to affect the expression of the *VDR* gene. The transcription factor called CDX-2 binds "A" allelic variants with the most powerful affinity, so the "AA" genotype of the VDR SNP (rs11568820, Cdx-2) allows binding to CDX-2 transcription factor with more affinity than other genotypes, causing increased *VDR* expression (Iqbal et al., 2015).

Over 400 SNPs have been detected in the *VDR* gene; however, the polymorphisms most prevalently studied in COVID-19 are Fok1 (Kotur et al., 2021; Apaydin et al., 2022; Abdollahzadeh et al., 2021), Taq1, Bsm I, Apa I (Apaydin et al., 2022; Abdollahzadeh et al., 2021), and Cdx-2 (Abdollahzadeh et al., 2021), with Cdx-2 being the least examined. Abdollahzadeh et al. (2021) investigated the association between Cdx-2 SNP (rs11568820) and COVID-19 in an Iranian population and found that the minor A allele was associated with decreased COVID-19 severity compared with major G allele. Until date, no information have been reported about the association of VDR-SNPs with COVID-19 vulnerability and severity in the Egyptian population.

This study showed an association between the VDR SNP (rs11568820, Cdx-2) with susceptibility to COVID-19 under the over-dominant model [heterozygotes ("AG") vs. homozygotes ("GG"+"AA")]. The heterozygous "AG" genotype emerges as consistently associated with decreased susceptibility (OR = 0.5, 95% CI = 0.28–0.9, $P = 0.019$) to COVID-19; "AG" genotype seems to have a protective effect against COVID-19 compared to "GG" and "AA" genotypes. The frequency of the "GG" genotype was significantly increased in COVID-19 patients vs. healthy controls.

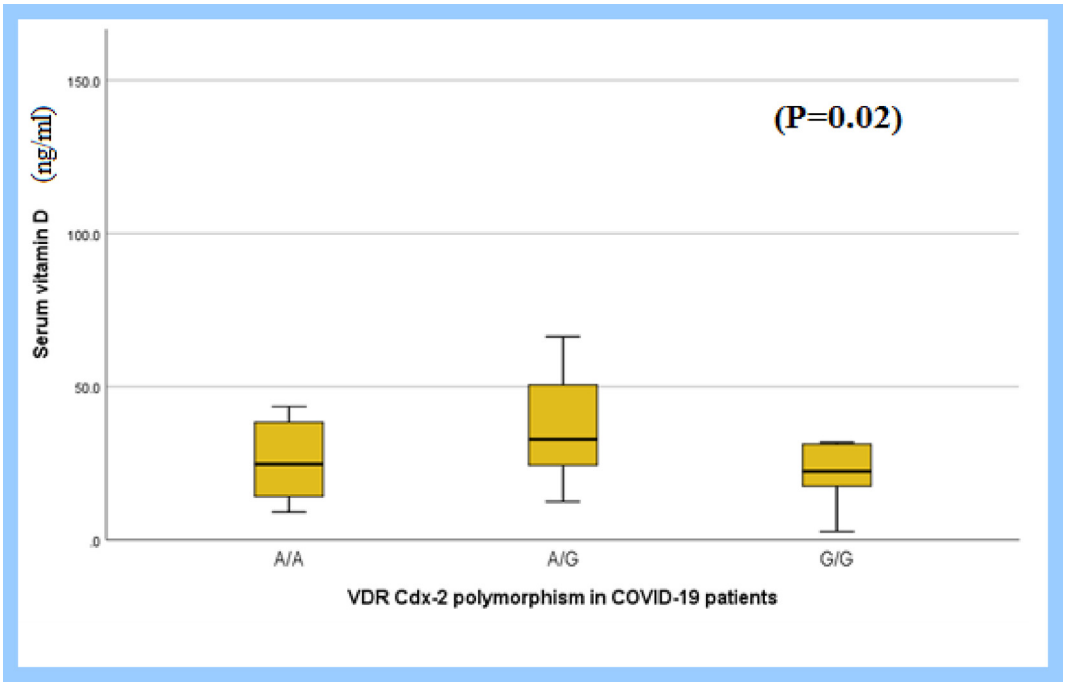

Fig. 2. Association between the VDR SNP (rs11568820, Cdx-2) and 25(OH)D levels among COVID-19 patients.

These results are in harmony with other studies that have shown that the "GG" genotype was associated with increased susceptibility to immune-dysregulated disorders. In a study performed on the Turkish population, the "GG" genotype of the VDR SNP (rs11568820, Cdx-2) was over-represented in patients with immune-thrombocytopenic purpura (Yesil et al., 2017). Dickinson et al. (2009) reported that the "G" allele was significantly associated with an increased risk of multiple sclerosis. The presence of the wild "G" allele at the binding site of CDX-2 transcription factor can diminish the transcriptional activity of the *VDR* primary promoter by 70% versus the mutant "A" allele, which could compromise the vitamin D action and mediate the immune imbalance (Arai et al., 2001; Selvaraj et al., 2008).

In contrast, the patients carried the "AA" genotype were associated with a more risk for tuberculosis infection in Indians (Alagarasu et al., 2009), while the heterozygous "AG" genotype of the VDR SNP (rs11568820, Cdx-2) was associated with increased vulnerability to HIV-1 infection (Maleki Dana et al., 2020). These controversial results are probably due to population genetic differences and that these infectious agents belong to different families with differences in the infection process.

Moreover, our analysis demonstrated that the protective effect of "AG" heterozygous genotype on COVID-19 and the effect of "GG" homozygous genotype in increasing the risk of COVID-19 appeared more significant among male than female participants ($P = 0.029$). That result confirms the validity of the hypothesis that the sex factor has a crucial role in the pathogenesis of COVID-19 (Hammad and Alseoudy, 2021) and more concern should be given to male patients with the 'GG' genotype as a risk group for COVID-19.

Our study found no significant differences in genotype or allele distributions for the VDR Cdx-2 polymorphism between the severe and nonsevere COVID-19 groups. These results agreed with those of Apaydin et al. (2022) who indicated that VDR polymorphisms are independently associated with the COVID-19 severity and the patients survival; however, these findings disagreed with those of Abdollahzadeh et al. (2021) who reported that the mutant minor "A" allele was found to be a predisposition factor to COVID-19 severity in which the frequency of the mutant minor "A" allele was higher in symptomatic and severe/critical patients against asymptomatic COVID-19 cases of Iranian population.

A combined analysis of serum vitamin D levels and VDR SNPs has been the most reasonable form of association investigation of many diseases. The exact mechanism of how this combination between genotypes and vitamin D level associated with disease predisposition is not known, possibly both decreased circulating vitamin D levels and certain genotypes in the *VDR* gene could significantly alter

the vitamin D signalling pathways (Ma et al., 2020). Meyer and Bornman (2018) had demonstrated that the effect of the VDR SNP (rs11568820, Cdx-2) on *VDR* gene expression depends on circulating vitamin D levels and VDR methylation and is devoid of CDX-2 transcription factor. Thus, we investigated the presence of a possible association between the three Cdx-2 SNP genotypes and 25(OH)D serum level.

Our results demonstrated that heterozygote "AG" were significantly associated with higher vitamin D levels than "GG" homozygote. This finding could help prevent the vitamin D deficiency by improving the dietary habits and monitoring the circulating 25(OH)D concentration of the patients with the risky "GG" genotype.

This finding disagreed with another study performed on middle-aged and elderly Chinese women, which found that the circulating 25(OH)D levels were highest in patients with "GG" genotype, lowest in patients with "AA" genotype, and in between patients with "AG" genotype, but in men, the Cdx-2 VDR SNP was not associated with circulating 25(OH)D levels (Ling et al., 2016). Moreover, another study observed no significant association between the Cdx-2 VDR SNP and serum 25(OH)D levels in polycystic ovary women (Wehr et al., 2011). This discrepancy might be explained by the ethnic variation of the VDR Cdx-2 SNP; and these two studies examined this association in a sex-dependent manner.

It is noteworthy that COVID-19 presentation and progression are heterogeneous among individuals and multiple genes could be involved in the pathogenesis of COVID-19 (Ovsyannikova et al., 2020). Because of the inter-individual disparity of COVID-19 and the complexity of the genetic factors, VDR genetic polymorphism cannot be the only reason responsible for the severity of COVID-19. Moreover, ethnic variations of VDR SNP (rs11568820, Cdx-2) may confound association studies linking VDR polymorphisms to disease. Hence, more related genes and different populations need to be included in future studies.

However, to the best of our knowledge, this study is the first one to examine the association between the VDR SNP (rs11568820, Cdx-2) and COVID-19 in Egyptians, and is considered an important step towards further future studies of this polymorphism with larger sample size.

4.1. Conclusion

This study concluded that the VDR Cdx-2 polymorphism may be a potential predisposing factor for COVID-19 susceptibility, especially among males. Nevertheless, we could not detect any significant association between the VDR Cdx-2 SNP and the severity of COVID-19. It cannot be excluded that this association may be present in other populations. A larger sample size in other populations is needed to be conducted to confirm the VDR role in determining COVID-19 severity.

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Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Ethics approval and consent to participate

This research was consistent with the Declaration of Helsinki and accepted by the Institutional Research Board (IRB) (approval: R.20.06.901). Written informed consents were obtained from all study contributors.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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