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FokI polymorphism of the vitamin D receptor gene: Linking COVID-19 risk to genetic susceptibility in children

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ABSTRACT

Background: Vitamin D receptor (VDR), influenced by gene polymorphisms like *FokI*, may affect susceptibility to infections, including coronavirus disease 2019 (COVID-19). Since studies in children are limited, we aimed to analyze the correlation between the VDR *FokI* variant and both the incidence and severity of COVID-19 in Egyptian children.

Methods: Seventy-seven COVID-19-positive and 107 COVID-19-negative pediatric patients were included. Participants' serum 25(OH)D levels, inflammatory biomarkers, and demographics were evaluated. Real-time polymerase chain reaction (PCR) was used for genotyping the VDR *FokI* (rs2228570) polymorphism.

Results: Absolute lymphocyte count (ALC) was significantly lower in COVID-19 patients than in controls, while interleukin-6 (IL-6), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), procalcitonin, and D-dimer were significantly higher (all $p < 0.001$). Vitamin D insufficiency was significantly more common in COVID-19

Abbreviations: ALC, Absolute lymphocyte count; ANC, Absolute neutrophil count; ARDS, Acute respiratory distress syndrome; BMI, Body mass index; CBC, Complete blood count; CI, Confidence intervals; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; CT, Computed tomography; ESR, Erythrocyte sedimentation rate; *HWEp*, Hardy–Weinberg equilibrium *p*-value; IL-6, Interleukin-6; INR, International normalized ratio; IQR, Interquartile range; OR, Odds ratio; PCR, Polymerase chain reaction; $p\text{-value}_{corr}$, corrected *p*-value; Ref, reference; RSV, Respiratory Syncytial Virus; RT-PCR, Reverse transcription polymerase chain reaction; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TLC, Total leucocytic count; TLRs, Toll-like receptors; TNF-α, Tumor necrosis factor-alph; VDR, Vitamin D receptor; WBCs, White blood cells; WHO, World Health Organization; χ2, Chi-squared value.

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cases (18.2 % versus 3.7 %, $p = 0.002$). Male sex, increased tumor necrosis factor-alpha (TNF-α), and CRP were significantly associated with severe COVID-19 ($p = 0.032$, 0.029, < 0.001, respectively). The *FokI* TT genotype in codominant and recessive models and the T allele in the multiplicative model were significantly correlated with 2.4, 3.0, and 1.8 folds increased COVID-19 risk ($p = 0.043$, < 0.001, and 0.004, respectively). However, VDR *FokI* variants did not significantly associate with severe COVID-19.

Conclusion: The T allele and TT genotype of the *FokI* variant in the *VDR* gene increase susceptibility to COVID-19 but not its severity in Egyptian children. Additional research is required to validate the potential role of vitamin D and its receptor polymorphism in COVID-19.

1. Introduction

The global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections still represents a serious clinical problem. Coronavirus disease 2019 (COVID-19) causes a range of manifestations involving minor respiratory symptoms, severe pneumonia with acute respiratory distress syndrome (ARDS), and possibly death [1,2]. Children with COVID-19 usually present with mild symptoms and are at lower risk of life-threatening complications [3]. Nevertheless, there have been reports of children having severe illness or a post-infectious multisystem inflammatory syndrome [4]. Thus, identifying risk variables linked to COVID-19 occurrence and COVID-19 severity in this age group is critical.

In this regard, vitamin D exhibits immunomodulatory, anti-inflammatory, antifibrotic, and antioxidant activities, besides its role in bone and calcium homeostasis health. Therefore, its deficiency leads to a variety of pathological diseases such as diabetes, cardiovascular disorders, cancer, respiratory infections, and autoimmune disorders [5–7]. Vitamin D deficiency is significantly linked to developing COVID-19-associated complications [8]. Furthermore, supplemental vitamin D prevents COVID-19-related complications such as multiorgan failure [9], coagulopathy [10], and death [11] while decreasing the severity of COVID-19 [12].

Research has concentrated on the VDR's role in preventing acute lung damage and ARDS, which developed in nearly 20 % of COVID-19 patients treated in hospitals [13]. The major mechanism of ARDS is the secretion of inflammatory cytokines, which is referred to as a cytokine storm [14]. Increasing evidence indicates that VDR signaling may significantly attenuate the cytokine storm and thus regulate various immune cells like neutrophils and monocytes/macrophages, thereby aiding in minimizing coagulation and thrombosis and maintaining the epithelial barrier of lungs [15].

The *VDR* gene exists on chromosome 12q13.11. The *VDR* gene polymorphism may impact VDR products' activity and expression levels, thereby altering the vitamin D-related signaling and disrupting its immune-regulatory functions. Several variants of *VDR* gene predispose to different conditions, though findings have been inconsistent, including *Cdx2* (rs11568820; G > A), *FokI* (rs2228570; C > T), *TaqI* (rs731236; A > G), *BsmI* (rs1544410; G > A), and *ApaI* (rs7975232; C > A) [16]. There is inconsistency in naming the alleles of these variants. Some authors utilized the initial of the variant's name in uppercase and lowercase (for ancestral and polymorphic alleles, respectively), whereas other authors used the first letter of the nucleotides implicated in the nucleotide sequence change [17]. Owing to the low prevalence of pediatric COVID-19 and the dispersed locations of the cases, current studies on this population are insufficient [18]. Therefore, our study aimed to examine the link between the *VDR* gene variant *FokI* and the incidence and severity of COVID-19 in Egyptian children.

2. Patients and methods

2.1. Patients and design

The study comprised a group of 77 pediatric patients (below 18 years) who had COVID-19 over one year. All patients' nasopharyngeal swabs were tested in the laboratory using the cobas 6800 reverse transcription polymerase chain reaction (RT-PCR) to verify SARS-CoV-2 infection presence. Additionally, another group of 107 participants who met the same criteria but tested negative for COVID-19 were included in the study. Only Egyptian inpatients and outpatients with no history of previous SARS-CoV-2 infections and no history of vaccination were included. We excluded participants with particular medical conditions, including immunodeficiency, obesity, malnutrition, congenital heart disease, autoimmune disorders, cancer, metabolic abnormalities, and any chronic sickness. In addition, individuals who had received vitamin D, multivitamins, or calcium supplementation within the last six months of the study were also excluded. Insufficiency of vitamin D was indicated by a serum 25(OH)D < 30 ng/mL [19].

2.2. Clinical and laboratory assessments

Full clinical history-taking, cardiac, neurological, respiratory examinations, and chest computed tomography (CT) scans were carried out for every participant. The COVID-19 patients' severity of illness was assessed following World Health Organization (WHO) criteria. Patients with mild COVID-19 exhibited non-specific symptoms and had normal chest CT. However, those with moderate to severe COVID-19 had pneumonia on CT, required oxygen, and/or showed signs of sepsis, organ dysfunction, and/or ARDS [20,21]. Body mass index (BMI) has been estimated by dividing one's body weight (kilograms) by the square height (meters) [22]. Laboratory investigations were performed for all participants, including inflammatory indices such as complete blood count (CBC), CRP, procalcitonin, ESR, TNF-α, and IL-6. Coagulation markers were also measured, including international normalized ratio (INR) and D-dimer. The 25(OH)D ELISA Kit (K2110 Immunodiagnostic) was used to measure the concentrations of serum 25(OH)D.

2.3. Genotyping for vitamin D receptor FokI polymorphism

Under aseptic conditions, approximately 3 mL of whole blood was collected from each participant. Genomic DNA was extracted by the QIAamp Blood Mini Kit (Qiagen). This extracted DNA was then preserved at −20 °C for genotyping. The VDR *FokI* (rs2228570, 27823C/T) variant was genotyped utilizing real-time PCR with the TaqMan® Universal PCR Master Mix and TaqMan® probes (Applied Biosystems) specific for each allele [23].

2.4. Statistical methods

Kolmogorov–Smirnov and Shapiro–Wilk tests analyzed the normality of numerical data. Qualitative data were presented as frequency and percentage, while median and interquartile range (IQR) represented the quantitive data. A comparison between qualitative variables was performed using Fisher's exact or Chi-square test. However, the correlation between numerical variables was analyzed using the Kruskal-Wallis or Mann-Whitney *U* test. The genotype and allele frequencies of COVID-19 and controls were compared using the Chi-square test after calculating the Hardy–Weinberg equilibrium. COVID-19 incidence and severity risk based on different genetic models of VDR were estimated and presented as odds ratios and 95 % confidence

intervals. Logistic regression analyses evaluated the independent effect of different *VDR FokI* genetic models on COVID-19 susceptibility and severity. A *p*-value ≤0.05 was considered to be statistically significant. Statistical analyses of data were done using IBM SPSS version 25.

3. Results

3.1. Demographics and laboratory testing of the studied cohorts

This study involved 77 COVID-19 patients and 107 healthy controls. Table 1 summarizes their demographic and laboratory characteristics. The median age for both groups was 11.0 years ($p = 0.131$). COVID-19 patients had a significantly higher BMI than controls ($p = 0.012$). Laboratory findings demonstrated significant variations between the two groups. The oxygen saturation levels, ALC, absolute neutrophil count (ANC), and total leucocytic count (TLC) were significantly lower, while levels of CRP, procalcitonin, ESR, IL-6, platelet count, INR, and D-dimer were significantly elevated in COVID-19 cases (*p*-value < 0.001 for all). However, TNF-α and vitamin D levels were not significantly different between groups. Vitamin D insufficiency was significantly more prevalent among COVID-19 patients than in control, while sufficient vitamin D was less common ($n = 14$, 18.2 % versus $n = 4$, 3.7 % and $n = 63$, 81.8 % versus $n = 103$, 96.3 %, respectively; $p = 0.002$). Regarding disease severity among the COVID-19 group, there were 57 patients with mild disease and 20 patients with moderate to severe disease (74.0 % and 26.0 %, respectively). Males predominated in the moderate to severe group (80.0 % versus 52.6 %, $p = 0.032$). Furthermore, the moderate to severe group demonstrated significantly higher CRP ($p = 0.029$) and TNF-α ($p < 0.001$) levels than the mild one, while other laboratory characteristics were comparable between the two severity groups.

3.2. Vitamin D receptor FokI variant and COVID-19 risk

Table 2 illustrates the distribution of different genetic models of the VDR *FokI* variant between COVID-19 cases and control groups. The control group was in equilibrium (Hardy–Weinberg equilibrium *p*-value (HWE*p*) = 0.237). The frequencies of the TT genotype in both codominant and recessive models and the T allele in the multiplicative model were significantly greater in COVID-19 cases ($p = 0.018$, 0.001, and 0.007, respectively). However, CT/TT genotypes in the dominant model and CT genotype in the codominant model were not significantly different between COVID-19 and controls ($p = 0.208$ and 0.538, respectively). Further binary logistic regression identified a significant correlation between the VDR *FokI* variant and the risk of COVID-19 (Fig. 1 and Supplementary Table S1). The TT genotype in the codominant and recessive models was significantly associated with increased COVID-19 occurrence ($p = 0.020$ and $p < 0.001$, respectively). T allele was also significantly linked to COVID-19 risk, with an adjusted OR of 1.766 and a *p*-value of 0.008. Demographic characteristics and laboratory findings of pediatric COVID-19 patients according to the VDR *FokI* variant genotypes are shown in Table 3. BMI was significantly higher in patients with TT genotype in comparison to those with CC and CT genotypes ($p < 0.001$), whereas oxygen saturation level was significantly lowered in TT and CT genotypes than the CC genotype ($p < 0.001$). Conversely, no significant differences were observed between genotype groups regarding age, sex, inflammatory markers, coagulation profile, and vitamin D levels.

Table 2
Distribution of the different genetic models of *vitamin* D receptor single nucleotide polymorphism (rs2228570 C/T) between children with and without COVID-19.

Genetic model	Genotype	Frequency (%)		χ2	*p-value*
		COVID-19 ($n = 77$)	Controls ($n = 107$)		
Codominant	CC	24 (31.2)	38 (35.5)	–	–
	CT	18 (23.4)	46 (43.0)	1.588	0.208
	TT	35 (45.5)	23 (21.5)	5.612	0.018*
	HWEp	–	0.237		
Dominant	CC	24 (31.2)	38 (35.5)	0.378	0.538
	CT/TT	53 (68.8)	69 (64.5)		
Recessive	CC/CT	42 (54.5)	84 (78.5)	11.908	0.001*
	TT	35 (45.5)	23 (21.5)		
Multiplicative (allele)	C	66 (42.9)	122 (57.0)	7.178	0.007*
	T	88 (57.1)	92 (43.0)		

χ2: Chi-squared value, *HWEp*: Hardy-Weinberg Equilibrium *p*-value for the control group. Asterisks (*) indicate statistically significant results ($p \leq 0.05$).

Table 1
Basic characteristics of pediatric patients according to COVID-19 presence and severity.

	Reference range	Median (IQR) or frequency		*p*-value	Median (IQR) or frequency		*p*-value
		Non COVID-19 ($n = 107$)	COVID-19 ($n = 77$)		Mild COVID-19 ($n = 57$)	Moderate to severe COVID-19 ($n = 20$)	
Demographic and clinical data							
Age (years)		11.0 (5.0)	11.0 (4.0)	0.131	11.0 (4.0)	11.0 (3.8)	0.694
Sex (male/female)		62/45	46/31	0.807	30/27	16/4	0.032*
BMI (kg/m^2)		20.0 (3.0)	22.0 (10.0)	0.012*	22.0 (10.0)	20.0 (8.8)	0.290
Oxygen saturation (%)	≥95	98.0 (1.0)	96.0 (3.0)	<0.001*	96.0 (2.5)	97.0 (3.5)	0.310
Inflammatory profile							
CRP (mg/L)	0–5	4.60 (2.5)	12.00 (14.8)	<0.001*	12.00 (7.5)	19.0 (30.5)	0.029*
Procalcitonin (ng/mL)	<0.1	0.24 (0.0)	0.50 (4.0)	<0.001*	0.45 (4.4)	0.56 (4.6)	0.907
ESR (mm/h)	0–10	10.0 (5.0)	12.0 (12.0)	<0.001*	12.0 (12.0)	12.0 (15.0)	0.981
Total WBCs (×10^9/L)	4.5–13	6.35 (4.0)	3.06 (2.0)	<0.001*	3.02 (2.0)	3.25 (2.0)	0.930
ANC (×10^9/L)	1.5–8	3.59 (2.4)	2.08 (1.1)	<0.001*	2.08 (1.1)	2.21 (1.2)	0.853
ALC (×10^9/L)	1–7	2.35 (1.8)	0.75 (0.4)	<0.001*	0.72 (0.5)	0.78 (0.2)	0.315
TNF-α (pg/mL)	2.2–3.5	29.00 (7.3)	24.50 (15.8)	0.405	23.00 (13.0)	38.50 (11.0)	<0.001*
IL-6 (pg/mL)	1.6–9.2	21.00 (6.0)	24.00 (13.8)	<0.001*	24.00 (13.9)	23.00 (12.5)	0.726
Coagulation and vitamin D							
Platelets count (×10^9/L)	150–450	215.5 (73.0)	295 (123.0)	<0.001*	300 (127.0)	270 (117.0)	0.762
INR	0.8–1.2	1.00 (0.0)	1.10 (0.0)	<0.001*	1.10 (0.0)	1.10 (0.0)	0.627
D-dimer (μg/mL)	<0.5	0.27 (0.0)	1.00 (1.0)	<0.001*	1.00 (0.0)	1.00 (1.0)	0.903
Vitamin D (ng/mL)	30–100	37.00 (7.0)	38.00 (13.0)	0.280	38.00 (10.0)	38.00 (14.0)	0.459

IQR: interquartile range, BMI: body mass index, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, WBCs: white blood cells, ANC: absolute neutrophil count, ALC: absolute lymphocyte count, TNF-α: tumor necrosis factor-alpha, IL-6: interleukin-6, INR: international normalized ratio. Asterisks (*) indicate statistically significant results ($p \leq 0.05$).

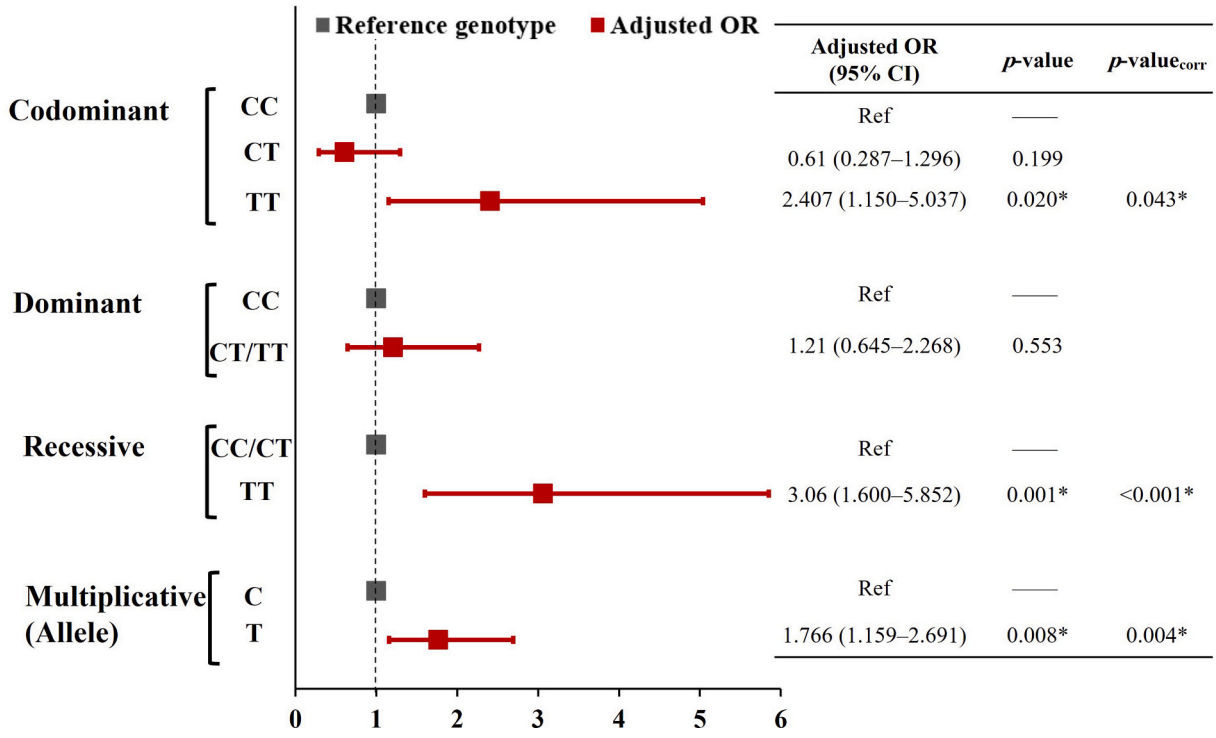

Fig. 1. Different genetic models of vitamin D receptor single nucleotide polymorphism (rs2228570 C/T) and COVID-19 risk in pediatric patients. OR: odds ratio, CI: confidence interval, p-value$_{corr}$: corrected p-value, Ref: reference. Asterisks (*) indicate statistically significant results ($p \leq 0.05$).

Table 3
Different genotypes of *vitamin* D receptor single nucleotide polymorphism (rs2228570 C/T) in relation to various characteristics of pediatric patients with COVID-19.

	Median (IQR) or frequency			p-value
	CC genotype ($n = 24$)	CT genotype ($n = 18$)	TT genotype ($n = 35$)	
Demographic and clinical data				
Age (years)	10.0 (3.8)	11.0 (3.5)	12.0 (4.0)	0.356
Sex (male/female)	15/9	10/8	21/14	0.901
BMI (kg/m^2)	20.5 (6.8)	20.0 (6.5)	30.0 (13.0)	<0.001*
Oxygen saturation	98.0 (2.0)	96.0 (2.0)	96.0 (2.0)	<0.001*
Inflammatory profile				
CRP (mg/L)	12.00 (20.0)	10.00 (7.0)	13.00 (17.0)	0.764
Procalcitonin (ng/mL)	0.45 (4.0)	3.60 (5.0)	0.44 (4.0)	0.167
ESR (mm/h)	13.0 (17.0)	13.0 (11.0)	10.0 (9.0)	0.663
Total WBCs (×10^9/L)	3.11 (2.0)	3.00 (1.0)	3.10 (1.0)	0.673
ANC (×10^9/L)	2.18 (1.2)	2.00 (1.1)	2.08 (0.7)	0.360
ALC (×10^9/L)	0.70 (0.6)	0.75 (0.3)	0.75 (0.3)	0.675
TNF-α (pg/mL)	24.00 (13.5)	25.00 (15.5)	23.00 (17.0)	0.835
IL-6 (pg/mL)	24.50 (13.8)	25.00 (14.0)	23.00 (14.0)	0.969
Coagulation and vitamin D				
Platelets count (×10^9/L)	300 (140.8)	290 (122.5)	271 (100.0)	0.402
INR	1.10 (0.0)	1.00 (0.0)	1.10 (0.0)	0.818
D-dimer (μg/mL)	1.00 (0.0)	0.91 (1.0)	1.00 (1.0)	0.751
Vitamin D (ng/mL)	39.00 (7.0)	34.00 (13.0)	35.00 (15.0)	0.475

IQR: interquartile range, BMI: body mass index, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, WBCs: white blood cells, ANC: absolute neutrophil count, ALC: absolute lymphocyte count, TNF-α: tumor necrosis factor-alpha, IL-6: interleukin-6, INR: international normalized ratio. Asterisks (*) indicate statistically significant results ($p \leq 0.05$).

3.3. Vitamin D receptor FokI variant and COVID-19 severity

COVID-19 patients were divided into two categories of disease severity: mild and moderate to severe. Table 4 shows the distribution of different genetic models of the VDR *FokI* variant between those groups. No statistically significant differences existed in the genotypes between patients with mild and moderate to severe diseases. Additionally, there were no significant variations in the risk ratio of distinct VDR *FokI* variant genetic models between the two groups (as shown in Fig. 2 and Supplementary Table S2).

4. Discussion

The WHO clarified the termination of the COVID-19 worldwide health emergency after analyzing the declining rates of COVID-19-related intensive care and hospital admissions and mortality.

Table 4
Distribution of the different genetic models of *vitamin* D receptor single nucleotide polymorphism (rs2228570 C/T) between children according to COVID-19 severity.

Genetic model	Genotype	Frequency (%)		χ2	p-value
		Mild cases ($n = 57$)	Moderate to severe cases ($n = 20$)		
Codominant	CC	18 (31.6)	6 (30.0)	–	–
	CT	13 (22.8)	5 (25.0)	0.041	0.839
	TT	26 (45.6)	9 (45.0)	0.004	0.951
Dominant	CC	18 (31.6)	6 (30.0)	0.017	0.896
	CT/TT	39 (68.4)	14 (70.0)		
Recessive	CC/CT	31 (54.4)	11 (55.0)	0.002	0.962
	TT	26 (45.6)	9 (45.0)		
Multiplicative (allele)	C	49 (43.0)	17 (42.5)	0.003	0.958
	T	65 (57.0)	23 (57.5)		

χ2: Chi-squared value. Asterisks (*) indicate statistically significant results ($p \leq 0.05$).

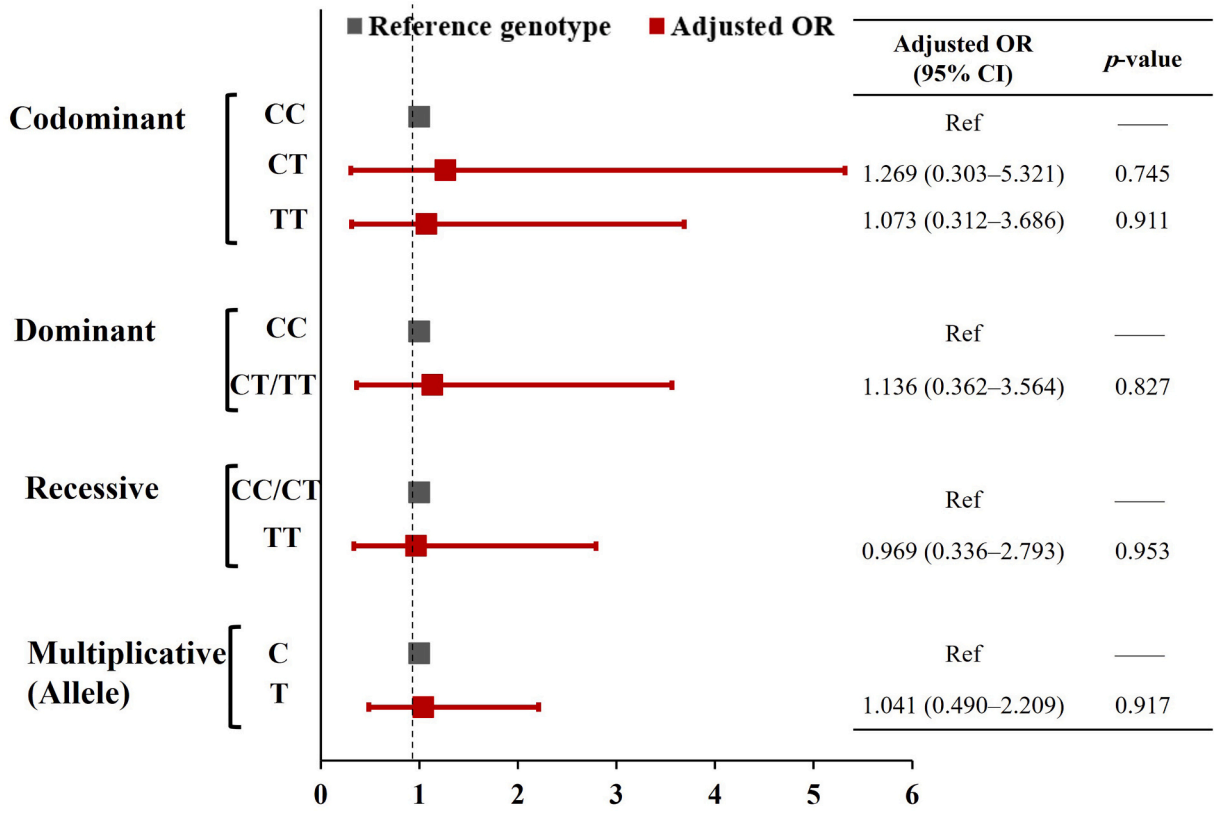

Fig. 2. Different genetic models of vitamin D receptor single nucleotide polymorphism (rs2228570 C/T) in pediatric patients and COVID-19 severity. OR: odds ratio, CI: confidence interval, Ref: reference.

However, the WHO administrator announced, "This virus is here to stay. It is still killing, and it is still evolving. There is still a possibility that new variants will emerge, causing new increases in cases and deaths. The worst thing a country could do is take this news to let down its guard, remove the procedures it already has built, or give the message to its citizens that COVID-19 is not a cause for concern anymore." [24]. As a result, moving from emergency to long-term management of COVID-19 alongside other infectious diseases is vital. In addition, it is important to understand various pathogenic pathways that may contribute to distinct COVID-19 severity levels.

Lymphopenia is a significant feature of SARS-CoV-2 infection with different possible mechanisms, such as direct bone marrow suppression, functional depletion of antiviral lymphocytes, and cytokine-mediated destruction of lymphocytes [25]. In our study, the median ALC in COVID-19 cases was 0.75×10^9/L, significantly lower in cases in comparison to the control group (p-value < 0.001). A similar finding was reported in COVID-19-infected pediatrics by Üstündağ et al. [26]. Additionally, the substantial relationship between lymphopenia and COVID-19 severity, complications, and even mortality was previously stated in many studies, especially in adult patients [25,27]. However, we did not reveal a statistically significant correlation between ALC and disease severity in our patients. Thus, lymphopenia is a vital hallmark of COVID-19 in pediatric patients.

In this study, levels of CRP, procalcitonin, ESR, total leucocytic count, IL-6, platelet count, INR, and D-dimer were significantly greater among children with COVID-19 in comparison to controls (all p-values $<$ 0.001). The severe immune response in SARS-CoV-2 infections, which is driven by both innate and adaptive immunity, leads to systemic inflammation, cytokine overproduction, and tissue damage. The dysregulated immune response, marked by elevated inflammatory markers, plays a critical role in disease severity and associated complications [15], explaining the significant differences in biomarkers between cases and controls. A significant link was found between elevated CRP and TNF-α levels and COVID-19 severity ($p = 0.029$ and < 0.001, respectively). These results highlight the significance of immune dysregulation and inflammation in COVID-19 pathogenesis, with CRP and TNF-α acting as potential markers for disease severity in children.

Vitamin D's antiviral action is a major topic of discussion in the global fight against COVID-19. Many mechanisms explain how vitamin D acts against SARS-CoV-2, including cathelicidin and defensins production [28]. Recent studies have shown that toll-like receptors (TLRs), especially TLR4, detect and activate the SARS-CoV-2 immune response. TLR4 plays a vital role in inflammatory events [29]. Vitamin D boosts immune responses by interacting with TLRs and increasing cathelicidin and β-defensin 2 production, which attracts immune cells to infected sites and enhances pathogen clearance [30,31]. Vitamin D also acts as a barrier against microbes and induces antimicrobial actions in the immune system, and this was observed in the lung epithelium [32].

Considerable evidence indicates that vitamin D3 plays a protective role against COVID-19. We could not observe significant discrepancies in median vitamin D values between cases and controls ($p = 0.280$) or between mild and severe COVID-19 cases ($p = 0.459$). However, vitamin D insufficiency was significantly more common in the cases compared to controls (18.2 % versus 3.7 %, $p = 0.002$). Similarly, vitamin D deficiency was significantly linked to COVID-19 risk [19], poor outcomes, and increased deaths in COVID-19 patients [33]. In another retrospective analysis in Switzerland, median serum vitamin D3 levels were remarkably different between SARS-CoV-2 positive and negative patients (11 ng/mL versus 24 ng/mL) [34]. Conversely, a Turkish study by Apaydin et al. [35] could not reveal significant variations in vitamin D values among COVID-19 severity groups. Thus, the immune response may be significantly impacted by vitamin D deficiency, which is essential in the pathogenesis of COVID-19. This highlights vitamin D as a modifiable risk factor with important public health implications for reducing the burden of infectious diseases like COVID-19.

Several VDR gene polymorphisms influence vitamin D function

through distinct mechanisms. The *FokI* (rs2228570) modifies the transcription start site, resulting in either a shorter, more active VDR protein or a longer, less efficient form. This variation influences immunological responses, perhaps increasing susceptibility to infections like COVID-19. Thus, *FokI*, unlike other VDR polymorphisms, directly alters the protein structure, potentially affecting vitamin D-mediated immune regulation [36]. Conversely, other polymorphisms, such as *BsmI*, *TaqI*, and *ApaI*, regulate mRNA stability, altering VDR expression without impacting protein structure. These variations can influence immune functions by altering the VDR expression levels without changing their protein structure, highlighting the complex role of VDR gene polymorphisms in immune responses and disease susceptibility [37]. A Respiratory Syncytial Virus (RSV) infection meta-analysis showed a high frequency of recessive TT alleles in *FokI* compared to the CT and CC genotypes. The T allele reduces the binding efficiency of the vitamin D3/VDR complex with target genes and increases RSV risk. This highlights a potential link between vitamin D3 and antiviral immune response [38]. The *FokI* polymorphism was also linked to susceptibility to community-acquired pneumonia in Egyptian children [39].

VDR polymorphisms, including *FokI*, *TaqI*, and *BsmI*, were linked to COVID-19 incidence and severity in many previous studies on adult patients [16,35,37,40]. Our study revealed that the TT genotype in both codominant (compared to the CC genotype) and recessive (compared to the combined CC + CT genotypes) models were significantly associated with 2.4 and 3.0 folds increased COVID-19 risk in the *FokI* polymorphism ($p = 0.043$ and < 0.001, respectively). These findings indicate that children carrying the TT genotype (two copies of the T allele) (homozygous form) are significantly more susceptible to COVID-19 compared to those carrying at least one C allele (CC or CT + CC). On the other hand, the dominant model (TT + CT compared to CC) did not show a significant link with COVID-19 susceptibility ($p = 0.553$), suggesting that a single copy of the T allele is insufficient to increase COVID-19 susceptibility in children. In addition, the T allele in the multiplicative (allelic) model (T compared to the C allele) significantly increased the risk of COVID-19 by 1.8 fold ($p = 0.004$), confirming an additive effect of the T allele. These findings suggest that the T allele of VDR *FokI* polymorphism, particularly in homozygous form, increases COVID-19 incidence in Egyptian children.

However, VDR *FokI* variants showed no significant association with or representing a risk for severe COVID-19. Constant with our findings, Zeidan et al. [19] in Egyptian children and Al Saba et al. [41] in Bangladeshi adults proved a significant relationship between *FokI* polymorphism and increased risk of COVID-19. But, they found no association between *FokI* polymorphism and COVID-19 severity. Alhammadin et al. also did not find a significant association between VDR *FokI* and the severity of COVID-19 or symptoms of long-term COVID-19 among Jordanian adults [42]. In addition, the *FokI* TT genotype and T allele were significantly linked to COVID-19 in adult patients from Cyprus and Bangladesh [41,43]. On the other hand, studies from Iran by Besharati et al. [44] and Greece by Tentolouris et al. [45] failed to confirm a significant association between *FokI* polymorphism and increased COVID-19 risk. These observations indicate that environmental and genetic factors may influence the connection between VDR polymorphisms and the risk and severity of COVID-19 across different populations.

A relatively small sample size is a limitation of this study, potentially affecting our findings' generalizability. Moreover, SARS-CoV-2 variants were not identified in our patients as sequencing techniques were not employed. In addition, we focused solely on the *FokI* polymorphism in the *VDR* gene, while other polymorphisms (e.g., *TaqI*, *ApaI*, *BsmI*) might also influence COVID-19 susceptibility and severity. Despite that, the study is one of the few that evaluated the correlation between VDR polymorphisms and COVID-19 in children, providing important insights into host genetics in COVID-19 susceptibility. Moreover, the study comprehensively evaluated a wide range of clinical and laboratory parameters, such as inflammatory markers besides genetic data, which allowed for a robust assessment of disease severity and immune response.

In conclusion, this study accentuates the role of immune dysregulation and inflammation in the pathogenesis of COVID-19 among Egyptian children. Severe infection is significantly related to male sex, elevated CRP, and TNF-α. A shift from urgent response to sustained care for COVID-19 is critical. Vitamin D insufficiency could strongly affect COVID-19 risk and severity. VDR *FokI* polymorphism, particularly the T allele and TT genotype, significantly influences susceptibility to infection, though its role in disease severity remains unproven. Future global databases and large-scale studies, including low- and middle-income countries, are required to confirm our findings and clarify the potential impact of vitamin D and its receptor polymorphism across diverse populations.

CRediT authorship contribution statement

Amal Ahmed Mohamed: Supervision, Methodology, Conceptualization. **Abdullah Taher Alanazi:** Writing – review & editing, Conceptualization. **Hoda H. Ahmed:** Data curation, Conceptualization. **Samar Elfiky:** Data curation, Conceptualization. **Muhammad T Abdel Ghafar:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ingy Maher:** Investigation, Conceptualization. **Sherin A. Taha:** Data curation, Conceptualization. **Mohammed Zakaria Ali AbuRahma:** Writing – review & editing, Conceptualization. **Waleed Elagawy:** Writing – review & editing, Conceptualization. **Dina A. Mohareb:** Investigation, Conceptualization. **Abeer M. Rawy:** Writing – review & editing, Conceptualization. **Heba M. Abostate:** Investigation, Conceptualization. **Amira AlSayed Youssef:** Investigation, Conceptualization. **Dalia Saeed Elsayed:** Writing – review & editing, Conceptualization. **Rasha M. Abdel-Hamid:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization.

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Ethics approval and consent to participate

Suez Canal University Faculty of Medicine's ethical committee authorized the study (Research 5308#) and fulfilled all the ethical aspects required in human research that comply with Helsinki's declaration. All participants' parents were assented after receiving full information about the study.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2025.156958.

Data availability

Data will be made available on request.

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