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RESEARCH ARTICLE

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Relationship between CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms on the vitamin D receptor gene with susceptibility to different SARS-CoV-2 variants

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Abstract

Several studies have revealed that vitamin D deficiency is linked to an increased risk of developing coronavirus disease 19 (COVID-19). In individuals with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, vitamin D receptor activation is required to decrease acute respiratory distress syndrome. The purpose of this study was to examine the genotypic distribution and allelic frequencies of CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms in COVID-19 patients with various SARS-CoV-2 variants. For genotyping of CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms, we used the polymerase chain reaction-restriction fragment length polymorphism technique in 1734 and 1450 recovered and deceased patients, respectively. The results indicated the rate of COVID-19 mortality was associated with CDX2 rs11568820 AA and GA genotypes in the Delta variant and with CDX2 rs11568820 AA in the Omicron BA.5 variant, while no association was shown in the Alpha variant. Therefore, the rate of COVID-19 mortality was associated with *EcoRV* rs4516035 TC and CC genotypes in the Delta variant, while no association was shown in the Alpha and Omicron BA.5 variants. According to our analysis, the T-G haplotype was more common in all SARS-CoV-2 variants. The C-A haplotype was associated with COVID-19 mortality in the Delta and Omicron BA.5 variants, and the T-A haplotype was related to the Alpha variant. In conclusion, the genotype frequencies of the CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms between SARS-CoV-2 variants were significantly different between the deceased patients and recovered patients. However, more studies should be done to confirm the results.

KEYWORDS

CDX2 rs11568820 and *EcoRV* rs4516035, COVID-19, SARS-CoV-2 variants

1 | INTRODUCTION

The World Health Organization (WHO) reports that in various regions, incidences of pneumonia with an unknown origin were observed in late 2019 and early 2020. The pathogen of this pneumonia, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as coronavirus infectious disease (COVID-19) (Gorbalenya et al., 2020; Zhou et al., 2020). Since the emergence of COVID-19 in December 2019, many SARS-CoV-2 mutations have been discovered. Because of its unique traits, the Delta variant was classified as a variant of concern (VOC) until November 2021. According to the Centers for Disease Control and

Prevention, VOC is a variant that attributes to higher transmissibility, a severe disease course, decreased treatment efficacy, and many other concerning features. The Omicron variant, also known as B.1.1.529, is a brand-new, severely mutated SARS-CoV-2 variant that WHO has now classified as a VOC on November 26, 2021 (Araf et al., 2022; Torjesen, 2021).

In several studies, vitamin D supplementation has been suggested as a viable method to lower the risk of COVID-19 infection and severity, although antiviral medication effects against SARS-CoV-2 remain to be discovered (Pal et al., 2021). However, studies on how adding cholecalciferol or calcifediol to a diet can affect COVID-19 results have led to conflicting findings, though some observational studies on hospitalized cases revealed that cholecalciferol or calcifediol supplementation resulted in decreased COVID-19 severity or mortality (Hernández et al., 2021; Ling et al., 2020).

Most of the vitamin D's biological effects are controlled by the high-affinity intracellular receptor known as the vitamin D receptor (VDR), functioning as a nuclear transcription factor and controlling the synthesis of proteins essential for bone mineral balance and cell growth. The *VDR* gene is 75 kb in size, has 11 exons, and is situated on chromosome 12. Exons 2 and 3 code for the amino acids that bind to DNA and exons 7, 8, and 9 are involved in the binding of vitamin D. In the *VDR* gene, numerous single-nucleotide polymorphisms (SNPs) can alter the gene expression and activation. The most extensively researched SNPs of this gene are *Fok*I (rs2228570; exon 2; C>T), *Bsm*I (rs1544410; intron 8; G>A), *Apa*I (rs7975232; intron 8; C>A), and *Taq*I (rs731236; exon 9; A>G) (Abdollahzadeh et al., 2021; Borgna-Pignatti et al., 2004).

Two important polymorphisms in the promoter region of *VDR* gene are CDX2 (rs11568820; promoter; G>A) and *Eco*RV or A-1012G/GATA (rs4516035; promoter; T>C), which have been shown to be associated with some diseases (Balta et al., 2018; Neamatallah et al., 2022).

There is little and insufficient information about the relationship between CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms and COVID-19 infection. Therefore, we sought to evaluate the potential relationship between the two above-mentioned SNPs located at the 5′-end of the *VDR* gene (CDX2 and *EcoRV*) and the severity of COVID-19 based on different SARS-CoV-2 variants in an Iranian population.

2 | MATERIALS AND METHODS

2.1 | Sample collection

This case–control study was conducted from November 2020 to February 2022 in Iran. Out of 14,117 patients referred to the university hospitals of Ilam University of Medical Sciences, 3184 patients were enrolled in this study in three peaks of COVID-19 infection (Alpha, Delta, and Omicron BA.5). Inclusion criteria were no previous infection with COVID-19, having Iranian nationality with the same ethnicity, willingness to participate in the study, and patients with positive COVID-19 confirmed by real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) method. Exclusion criteria were patients with any underlying disease such as heart, lung, liver and kidney diseases, diabetes, pregnancy, taking immunosuppressive drugs, cancer, hepatitis viruses, and human immunodeficiency virus were selected.

Our study included adult patients with mild and moderate infection who eventually recovered and patients with severe and critical symptoms of COVID-19 who unfortunately died, as defined by the WHO criteria for clinical care of COVID-19 (World Health Organization, 2021). In this study, due to the lack of access to people who did not have a history of COVID-19 disease, we considered "recovered patients" as "controls" and "deceased patients" as "cases" and compared the results between these two groups.

All clinical parameters of the patients such as liver enzymes, lipid profiles, 25-hydroxyvitamin D, real-time PCR cycle threshold (C_t) values, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), uric acid, creatinine, and complete blood count (CBC) were collected at the time of admission.

2.2 | CDX2 rs11568820 and *EcoRV* rs4516035 genotyping

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to investigate the CDX2 rs11568820 and *EcoRV* rs4516035. All individuals' blood samples were taken in an EDTA tube, and genomic DNA was isolated from peripheral blood leukocytes using the High-pure PCR Template Preparation Kit (Roche Diagnostics Deutschland GmbH).

The CDX2 rs11568820 primers, F-(5′-AGGAGGGAGGGAG GAAGG-3′) and R-(5′-TGAGAGACATGAGCGTGGAG-3′), were used to amplify a fragment of 414 base pairs (bp). Also, a 181 bp fragment of *EcoRV* rs4516035 was amplified from the isolates using primers F-(5′-GAGGACAGGTGAAAAAGATGGGGTTC-3′) and R-(5′- CCTC CTCTGTAAGAGGCGAATAGCGAT-3′), as previously described (Abdollahzadeh et al., 2021). The PCR condition for CDX2 rs11568820 was as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 61°C for 30 s, 72°C for 30 s, and final extension at 72°C for 10 min. The PCR condition for *EcoRV* rs4516035 was as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 68°C for 30 s, 72°C for 30 s, and final extension at 72°C for 10 min.

The PCR products were digested with *HpyCH4III* and *EcoRV* restriction enzymes (Fermentas). The RFLP product was observed by electrophoresis on a 2.5% agarose gel after incubation. For CDX2 rs11568820, the product sizes for GG genotype were 254 bp, 110 bp, and 50 bp and 254 bp and 160 bp were for the AA genotype and 154 bp and 27 bp were for the TT genotype and 181 bp was for the CC genotype in *EcoRV* rs4516035 (Abdollahzadeh et al., 2021). To corroborate the PCR-RFLP results, at least 10% of the samples were randomly genotyped using the Sanger sequencing method.

2.3 | Statistical analyses

The SPSS program was used for all statistical analysis (version 22.0; SPSS, Inc.). Categorical data were expressed as frequency and percentage (%), whereas continuous data were expressed as mean ± standard deviation (SD). To evaluate if numerical variables follow a normal distribution, the Shapiro–Wilk test was used. The chi-square test or Fisher exact test was used to compare categorical data. The comparison of the continuous data was made using the Mann–Whitney *U* test analysis. The level of significance in all tests was considered to be less than 0.05.

The Hardy-Weinberg equilibrium (HWE) and the relationship between CDX2 rs11568820 and *EcoRV* rs4516035 and COVID-19 infection, as well as the minor allele frequency (MAF) and associations between these SNPs and COVID-19 mortality, under four inheritance models (dominant, codominant, overdominant, and recessive) were evaluated using SNPStats software. The Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were employed to determine the best model (https://www.snpstats.net/). The odds ratio (OR) and associated 95% confidence intervals (CIs) were used to determine the strength of association.

3 | RESULTS

3.1 | Clinical and demographic characteristics

Table 1 summarizes the demographic and clinical characteristics of the subjects. Overall, 3184 patients were included in this trial, of whom 1022 patients were infected with the Alpha variant, 1026 patients with the Delta variant, and 1132 patients with the Omicron BA.5 variant. The mean age of patients with the Alpha, Delta, and Omicron BA.5 variants was 53.0 ± 12.7, 58.0 ± 11.8, and 53.7 ± 12.9, respectively. The frequency of males and females was 479 (46.9%) and 543 (53.1%) in patients with the Alpha variant, and 546 (53.2%) and 480 (46.8%) in patients with the Delta variant, and 546 (53.2%) and 480 (46.8%) in patients with the Omicron BA.5, respectively.

Patients infected with Delta variant (21.8 ± 10.3) had a lower value of 25-hydroxy vitamin D than the Alpha (24.2 ± 12.8) and Omicron BA.5 (33.0 ± 13.4) variants ($p = .029$). The mean values for qPCR Ct for the Omicron BA.5 variant (21.9 ± 6.0) was higher than the Alpha (20.1 ± 6.4) and Delta (17.4 ± 6.1) variants ($p < .001$).

3.2 | CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms' frequency among SARS-CoV-2 variants

In our study, the highest mortality rate was seen in the Delta variant compared to the Alpha and Omicron BA.5 variants ($p < .001$). There were 501 (49.0%) of GG genotypes, 389 (38.1%) of GA genotypes, and 132 (12.9%) of AA genotypes in the Alpha variant of the CDX2 rs11568820 polymorphism. There were 161 (15.7%), 499 (48.6%), and 366 (35.7%) of each of these genotypes, respectively, in the Delta variant, as well as 481 (42.3%), 539 (47.4%), and 116 (10.3%) in the Omicron BA.5 variant (Table 1).

After adjusting for CDX2 rs11568820 genotypes and SARS-CoV-2 variants, the rate of COVID-19 mortality was associated with CDX2 rs11568820 AA (OR: 5.99, 95% CI: 3.99–8.98) and GA (OR: 5.58, 95% CI: 3.79–8.21) genotypes in the Delta variant and with CDX2 rs11568820 AA (OR: 4.17, 95% CI: 2.73–6.36) in the Omicron BA.5 variant, while no association was shown in the Alpha variant (Table 2).

There were 461 (45.1%) of TT genotypes, 524 (51.3%) of TC genotypes, and 37 (3.6%) of CC genotypes in the Alpha variant of the *EcoRV* rs4516035 polymorphism. There were 378 (36.8%), 530 (51.7%), and 118 (11.5%) of each of these genotypes, respectively, in the Delta variant, as well as 571 (50.3%), 496 (43.7%), and 69 (6.0%) in the Omicron BA.5 variant (Table 1).

After adjusting for *EcoRV* rs4516035 genotypes and SARS-CoV-2 variants, the rate of COVID-19 mortality was associated with *EcoRV* rs4516035 TC (OR: 10.04, 95% CI: 7.32–13.76) and CC (OR: 7.70, 95% CI: 4.66–12.72) genotypes in the Delta variant, while, no association was shown in the Alpha and Omicron BA.5 variants (Table 2).

According to our analysis, the T-G haplotype was more common in all SARS-CoV-2 variants. The C-A haplotype was associated with COVID-19 mortality in the Delta (OR: 5.09, 95% CI: 3.86–6.54) and Omicron BA.5 (OR: 0.50, 95% CI: 0.36–0.69) variants, and the T-A haplotype was associated with the Alpha variant (OR: 2.69, 95% CI: 1.69–4.26) (Table 4).

3.3 | Association between CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms and COVID-19 mortality according to SARS-CoV-2 variants

The COVID-19 mortality rate was considerably greater in patients with the CDX2 rs11568820 AA genotype than in patients with other genotypes. Patients with the CC genotype showed a higher COVID-19 mortality rate in the *EcoRV* rs4516035 polymorphism. Recovered patients with COVID-19 also had the *EcoRV* rs4516035 TT genotype.

The results of genetic model analysis of CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms are indicated in Table 3. The best-fitting models for CDX2 rs11568820 and *EcoRV* rs4516035 were recessive and codominant inheritance models with the lowest AIC and BIC values. The genotype AA of CDX2 rs11568820 ($p < .0001$, OR: 2.06, 95% CI: 1.69–2.51) and CC genotype of *EcoRV* rs4516035 ($p < .0001$, OR: 5.74, 95% CI: 4.12–7.99) were linked to a higher risk of COVID-19 mortality.

HWE in the CDX2 rs11568820 polymorphism agreed with both recovered and deceased patients ($p > .05$), but in the *EcoRV* rs4516035 polymorphism, did not agree. In deceased patients, the MAF for CDX2 rs11568820 (A) and *EcoRV* rs4516035 (C) polymorphisms was higher in deceased patients than in recovered patients.

TABLE 1 Comparison of laboratory parameters between SARS-CoV-2 variants.

Variables	SARS-CoV-2 variants			p Value
	Alpha (*n* = 1022)	Delta (*n* = 1026)	Omicron BA.5 (*n* = 1136)	
Deceased/recovered patients	479/543 (46.9/53.1%)	674/352 (65.7/34.3%)	297/839 (26.1/73.9%)	<.001*
Mean age ± SD	53.0 ± 12.7	58.0 ± 11.8	53.7 ± 12.9	.128
Gender (male/female)	525/497 (51.4/48.6%)	546/480 (53.2/46.8%)	598/538 (52.6/47.4%)	.692
ALT, IU/L (mean ± SD) (Reference range: 5–40)	38.5 ± 24.8	40.8 ± 24.7	35.8 ± 24.2	.001
AST, IU/L (mean ± SD) (Reference range: 5–40)	34.9 ± 15.5	34.5 ± 14.0	31.9 ± 14.4	<.001*
ALP, IU/L (mean ± SD) (Reference range: up to 306)	190.2 ± 84.7	188.6 ± 74.0	177.2 ± 83.5	<.001*
Cholesterol, mg/dL (mean ± SD) (Reference range: 50–200)	116.1 ± 34.1	120.5 ± 40.5	123.1 ± 39.4	<.001*
TG, mg/dL (mean ± SD) (Reference range: 60–165)	124.1 ± 54.9	121.6 ± 48.8	126.9 ± 55.9	.245
LDL, mg/dL (mean ± SD) (Reference range: up to 150)	82.8 ± 45.1	85.3 ± 45.3	104.7 ± 48.3	<.001*
HDL, mg/dL (mean ± SD) (Reference range: >40)	32.5 ± 11.3	32.1 ± 11.5	33.6 ± 11.7	.039*
WBC, 10^9/L (mean ± SD) (Reference range: 4000–10,000)	7627.3 ± 2843.2	7599.2 ± 2715.7	7704.9 ± 2807.7	.297
CRP, mg/L (mean ± SD) (Reference range: <10 mg/L negative)	61.6 ± 21.5	63.9 ± 22.0	60.2 ± 21.7	.122
ESR, mm/1st h (mean ± SD) (Reference range: 0–15)	50.1 ± 16.0	52.3 ± 16.0	49.1 ± 16.1	.025
FBS, mg/dL (mean ± SD) (Reference range: 70–100)	107.1 ± 41.6	109.8 ± 43.2	106.5 ± 40.7	.716
Platelets × 1000/cumm (mean ± SD) (Reference range: 140,000–400,000)	184 ± 71	185 ± 74	184 ± 69	.994
Uric acid, mg/dL (mean ± SD) (Reference range: 3.6–6.8)	4.8 ± 1.8	4.4 ± 1.7	5.2 ± 1.8	<.001*
Creatinine, mg/dL (mean ± SD) (Reference range: 0.6–1.4)	0.9 ± 0.3	1.0 ± 0.3	0.8 ± 0.3	<.001*
qPCR C_t value	20.1 ± 6.4	17.4 ± 6.1	21.9 ± 6.0	<.001*
25-hydroxy vitamin D, ng/mL (mean ± SD) (Sufficiency: 21–150)	24.2 ± 12.8	21.8 ± 10.3	33.0 ± 13.4	.029*
CDX2 rs11568820				<.001*
GG	501 (49.0%)	161 (15.7%)	481 (42.3%)	
GA	389 (38.1%)	499 (48.6%)	539 (47.4%)	
AA	132 (12.9%)	366 (35.7%)	116 (10.3%)	
EcoRV rs4516035				<.001*
TT	461 (45.1%)	378 (36.8%)	571 (50.3%)	
TC	524 (51.3%)	530 (51.7%)	496 (43.7%)	
CC	37 (3.6%)	118 (11.5%)	69 (6.0%)	

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FBS, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; TG, triglyceride; VDR, vitamin D receptor; WBC, white blood cells.

*Statistically significant (<0.05).

3.4 | Factors related to COVID-19 mortality

According to the multivariate logistic regression model, the COVID-19 mortality rate was related to mean age (OR: 0.932, 95% CI: 0.914–0.950, $p < .001$), ALT (OR: 0.979, 95% CI: 0.969–0.988, $p < .001$), HDL (OR: 1.028, 95% CI: 1.010–1.047, $p = .003$), LDL (OR: 1.017, 95% CI: 1.012–1.023, $p < .001$), FBS (OR: 0.993, 95% CI: 0.988–0.998, $p = .010$), uric acid (OR: 2.042, 95% CI: 1.757–2.373, $p < .001$), creatinine (OR: 0.079, 95% CI: 0.041–0.153, $p < .001$), ESR (OR: 0.973, 95% CI: 0.960–0.987, $p < .001$), 25-hydroxyvitamin D (OR: 1.030, 95% CI: 1.011–1.049, $p = .002$), real-time PCR C_t values (OR: 2.057, 95% CI: 1.896–2.233, $p < .001$), SARS-CoV-2 variants (OR: 2.545, 95% CI: 1.946–3.330, $p < .001$), CDX2 rs11568820 (OR: 2.195, 95% CI: 1.435–3.358, $p < .001$), and *EcoRV* rs4516035 (OR: 0.520, 95% CI: 0.308–0.879, $p = .015$) (Table 5).

TABLE 2 CDX2 rs11568820 and *EcoRV* rs4516035 genotypes association with SARS-CoV-2 variants.

Variants	rs11568820 Genotypes	Recovered patients	Deceased patients	OR (95% CI)
Alpha	G/G	224	277	1.00
	G/A	258	131	0.76 (0.64–1.00)
	A/A	61	71	0.94 (0.64–1.38)
Delta	G/G	111	50	1.00
	G/A	142	357	5.58 (3.79–8.21)
	A/A	99	267	5.99 (3.99–8.98)
Omicron BA.5	G/G	359	122	1.00
	G/A	432	107	0.72 (0.54–1.02)
	A/A	48	68	4.17 (2.73–6.36)
Variants	**rs4516035 genotypes**	**Recovered patients**	**Deceased patients**	**OR (95% CI)**
Alpha	T/T	193	268	1.00
	T/C	326	198	-
	C/C	24	13	-
Delta	T/T	246	132	1.00
	T/C	83	447	10.04 (7.32–13.76)
	C/C	23	95	7.70 (4.66–12.72)
Omicron BA.5	T/T	571	0	1.00
	T/C	250	246	-
	C/C	18	51	-

Abbreviations: CI, confidence intervals; OR, odds ratios; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VDR, vitamin D receptor.

4 | DISCUSSION

We examined CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms on the *VDR* gene in a cohort of 3184 Iranian patients positive for SARS-COV-2 infection (including 1450 deceased patients and 1734 recovered subjects) to evaluate any possible links between these SNPs and the susceptibility to SARS-CoV-2 variants and the clinical outcome of the disease.

In this study, the MAF for CDX2 rs11568820 (A) and *EcoRV* rs4516035 (C) was 0.36 and 0.31, which were related to high COVID-19 mortality.

MAF for CDX2 rs11568820 has been reported in other populations with different ethnicities by the National Center for Biotechnology Information (NCBI). (https://www.ncbi.nlm.nih.gov/snp/rs11568820). For example in our study, MAF was comparable to other population in Asian (0.395), East Asian (0.387), South Asian (0.328), other Asian (0.450), and Latin American (0.370), but not to European (0.198), African (0.798), and African American (0.794). The deceased cases (0.49) had a higher frequency of the CDX2 rs11568820 (A) allele than recovered ones (0.36).

MAF for *EcoRV* rs4516035 has been reported in other populations with different ethnicities in the NCBI (https://www.ncbi.nlm.nih.gov/snp/rs4516035). For example, in our study, MAF was comparable to Iran and other population in South Asian (0.261), Latin American (0.339), and European (0.425), but not to African (0.092), Asian (0.028), East Asian (0.020), other Asian (0.065), and African American (0.095). The deceased cases (0.42) had a higher frequency of the *EcoRV* rs4516035 (C) allele than recovered ones (0.23).

In this study, the highest mortality rate was found in the Delta variant. Interestingly, the serum level of vitamin D in these patients was lower than the patients with the other two variants, which was statistically significant. Vitamin D boosts cellular innate immunity in part by inducing antimicrobial peptides, such as human cathelicidins and defensins. Cathelicidins have direct antibacterial properties against a wide range of microorganisms, such as numerous species of bacteria, nonenveloped and enveloped viruses, and fungi. Additionally, it works by boosting the levels of anti-inflammatory cytokines and decreasing the levels of proinflammatory cytokines, causing inflammation and damage to the lung's lining during viral infections (such as COVID-19) and leading to pneumonia (Grant et al., 2020). Although vitamin D insufficiency is a global issue, it is more prominent in the elderly, who are more at risk for developing severe COVID-19 infection. One of the main contributing causes of severe

TABLE 3 CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms association with COVID-19 mortality adjusted by SARS-CoV-2 variants.

CDX2 rs11568820		Groups					
Model	Genotype	Recovered patients	Deceased patients	OR (95% CI)	*p* Value	AIC	BIC
Allele	G	2220 (64.0%)	1493 (51.0%)	-	-	-	-
	A	1248 (36.0%)	1407 (49.0%)	-	-	-	-
Codominant	G/G	694 (40.0%)	449 (31.0%)	1.00	<.0001*	3994.0	4024.3
	G/A	832 (48.0%)	595 (41.0%)	0.92 (0.78–1.09)			
	A/A	208 (12.0%)	406 (28.0%)	1.96 (1.57–2.45)			
Dominant	G/G	694 (40.0%)	449 (31.0%)	1.00	.15	4043.7	4068.0
	G/A-A/A	1040 (60.0%)	1001 (69.0%)	1.12 (0.96–1.32)			
Recessive	G/G-G/A	1526 (88.0%)	1044 (72.0%)	1.00	<.0001*	3992.9	4017.1
	A/A	208 (12.0%)	406 (28.0%)	2.06 (1.69–2.51)			
Overdominant	G/G-A/A	902 (52.0%)	855 (59.0%)	1.00	<.0001*	4027.5	4051.7
	G/A	832 (48.0%)	595 (41.0%)	0.72 (0.62–0.84)			
Minor allele frequency (A)		0.36	0.49	-	-	-	-
***EcoRV* rs4516035**							
Allele	T	2679 (77.0%)	1691 (58.0%)	-	-	-	-
	C	798 (23.0%)	1209 (42.0%)	-	-	-	-
Codominant	T/T	1010 (58.2%)	400 (27.6%)	1.00	<.0001*	3768.1	3798.5
	T/C	659 (38.0%)	891 (61.4%)	3.39 (2.89–3.99)			
	C/C	65 (3.8%)	159 (11.0%)	5.74 (4.12–7.99)			
Dominant	T/T	1010 (58.2%)	400 (27.6%)	1.00	<.0001*	3776.6	3800.8
	T/C-C/C	724 (41.8%)	1050 (72.4%)	3.60 (3.08–4.22)			
Recessive	T/T-T/C	1669 (96.2%)	1291 (89.0%)	1.00	<.0001*	3997.2	4021.5
	C/C	65 (3.8%)	159 (11.0%)	2.95 (2.15–4.05)			
Overdominant	T/T-C/C	1075 (62.0%)	559 (38.5%)	1.00	.371	3884.6	3908.9
	T/C	659 (38.0%)	891 (61.4%)	2.64 (2.27–3.07)			
Minor allele frequency (C)		0.23	0.42	-	-	-	-

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; COVID-19, coronavirus disease; CI, confidence intervals; OR, odds ratios; VDR, vitamin D receptor.

*Statistically significant (<0.05).

Haplotypes	Frequency	Alpha OR (95% CI)	Delta OR (95% CI)	Omicron OR (95% CI)
TG	0.5534	1.00	1.00	1.00
CA	0.2841	-	5.02 (3.86–6.54)	21.01 (12.82–34.44)
CG	0.0297	-	-	-
TA	0.1329	2.69 (1.69–4.26)	-	1.12 (0.61–2.05)

TABLE 4 SARS-CoV-2 variants and CDX2 rs11568820 and *EcoRV* rs4516035 haplotype.

Abbreviations: CI, confidence intervals; OR, odds ratios; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNPs, single nucleotide polymorphisms; VDR, vitamin D receptor.

TABLE 5 Factors associated with deceased patients infected with COVID-19.

Factors Baseline predictors	OR (95% CI)	*p* Value
Mean age ± SD	0.932 (0.914–0.950)	<.001*
ALT, IU/L	0.979 (0.969–0.988)	<.001*
HDL, mg/dL	1.028 (1.010–1.047)	.003*
LDL, mg/dL	1.017 (1.012–1.023)	<.001*
FBS, mg/dL	0.993 (0.988–0.998)	.010*
Uric acid, mg/dL	2.042 (1.757–2.373)	<.001*
Creatinine, mg/dL	0.079 (0.041–0.153)	<.001*
ESR, (mm/1st h)	0.973 (0.960–0.987)	<.001*
25-hydroxyvitamin D, (ng/Ml)	1.030 (1.011–1.049)	.002*
Real-time PCR C_t values	2.057 (1.896–2.233)	<.001*
SARS-CoV-2 variants	2.545 (1.946–3.330)	<.001*
CDX2 rs11568820	2.195 (1.435–3.358)	<.001*
EcoRV rs4516035	0.520 (0.308–0.879)	.015*

Abbreviations: C_t, cycle threshold; CI, confidence intervals; ESR, erythrocyte sedimentation rate; FBS, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LT, alanine aminotransferase; OR, odds ratios; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; VDR, vitamin D receptor.

*Statistically significant (<0.05).

COVID-19 infections is the secretion of proinflammatory cytokines. However, vitamin D controls its level in the body by limiting the amount of proinflammatory cytokines and chemokines released by macrophages. Therefore, it should not be surprising that several studies found a link between vitamin D deficiency and COVID-19 cases and a higher risk of death (Ahmadi et al., 2022; Gholami et al., 2022; Ilie et al., 2020; Khalilzadeh et al., 2022; Mirzaei Gheinari et al., 2022; Raheem Juhi Al-Kaabi et al., 2022; Rahimi et al., 2021).

The polymorphisms in the *VDR* gene have been shown to be related to the COVID-19 disease, and some of them have different effects according to the various SARS-CoV-2 variants (Albu-Mohammed et al., 2022).

The COVID-19 mortality rate was considerably greater in patients with the CDX2 rs11568820 AA genotype than in patients with other genotypes. Also, the rate of COVID-19 mortality was associated with CDX2 rs11568820 AA and GA genotypes in the Delta variant and with CDX2 rs11568820 AA genotype in the Omicron BA.5 variant, while no association was found in the Alpha variant in this study. Transcription factor CDX2 has a functional binding site in the 1a promoter region of the *VDR* gene called the CDX2 site. It has been discovered that the CDX2 rs11568820 G to A substitution polymorphism at this location affects how the *VDR* gene is transcribed, with the A-allele increasing binding to the CDX2 protein and the transcriptional activity of the *VDR* promoter compared to the G allele (Fang et al., 2003). According to the findings of Abdollahzadeh et al. the frequency of the A minor allele was higher in symptomatic and severe COVID-19 individuals than in asymptomatic individuals, while the prevalence of the G major allele was lower. Therefore, A and G alleles can be described as risk and protective factors, respectively (Abdollahzadeh et al., 2021). The CDX2 rs11568820 polymorphism of the *VDR* gene is regarded as a functional polymorphism since it affects the immune system and alters the risk of developing certain infectious diseases such as tuberculosis, hepatitis C virus, and rubella (Meyer & Bornman, 2018, Neamatallah et al., 2022, Ovsyannikova et al., 2010).

Patients with the CC genotype showed a higher COVID-19 death rate in the *EcoRV* rs4516035 polymorphism. Recovered patients with COVID-19 also have the *EcoRV* rs4516035 TT genotype. Therefore, the rate of COVID-19 mortality was associated with *EcoRV* rs4516035 TC and CC genotypes in the Delta variant, while no association was found in the Alpha and Omicron BA.5 variants. Similar to the CDX2 rs11568820 polymorphism, the *EcoRV* rs4516035 polymorphism is located in the *VDR* gene's promoter region. This polymorphism is thought to play a role in the immune response against cancer. SNPs in the regulatory region known as *EcoRV* (5′ to exon 1a) have the potential to influence VDR transcription through differences in transcription factor binding (Halsall et al., 2004). It was found that the frequency of the *EcoRV* rs4516035 C allele was much more likely to increase in severe COVID-19 patients compared to asymptomatic COVID-19 patients, whereas the rate of the *EcoRV* rs4516035 T allele was lower (Abdollahzadeh et al., 2021). According to earlier studies, *EcoRV* rs4516035 is linked to diabetes and human immunodeficiency virus-1 susceptibility (Ghodsi et al., 2021; Halsall et al., 2004).

The T-G haplotype was more common in all SARS-CoV-2 variants. The C-A haplotype was associated with COVID-19 mortality in the Delta and Omicron BA.5 variants, and the T-A haplotype was associated with the Alpha variant. It seems that these CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms could play a role in the control and severity of COVID-19 infection, but the results showed that they had various behaviors with an unknown mechanism compared to different SARS-CoV-2 variants. The superiority of our study over other studies in this field is that we evaluated the relationship of these polymorphisms on different SARS-CoV-2 variants, while other studies have only examined their relationship with the severity and the control of COVID-19.

In addition to strengths, this study also had limitations. One of the most important limitations of this study is the lack of access to a healthy control group that had not yet been infected with COVID-19. In addition, our information about vaccination in patients was not complete. Also, this study was conducted in one ethnic group in Iran, and more studies should be conducted with different ethnic groups to confirm our results.

5 | CONCLUSION

Our findings showed that the genotype frequencies of the CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms were significantly different between deceased and recovered patients with different

SARS-CoV-2 variant infections. The findings also imply that individuals with MAF of CDX2 rs11568820 and *EcoRV* rs4516035 polymorphisms might be more likely to contract the SARS-CoV-2 Delta and Omicron BA.5 variants.

AUTHOR CONTRIBUTIONS

Abed K. K. Al-Mohammedawi: Investigation; methodology; writing—original draft. **Enayat Anvari**: Data curation; formal analysis; investigation; writing—original draft. **Abolfazl Fateh**: Formal analysis; project administration; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The Ethics Committee of Ilam University of Medical Sciences granted approval for this study (IR.MEDILAM.REC.I400.237). The Declaration of Helsinki was followed when conducting the study. Moreover, written informed consent was directly obtained from all participants.

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