Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Archives of Medical Research

Archives of Medical Research xxx (xxxx) xxx

ORIGINAL ARTICLE

Role of *FokI* rs2228570 and *Tru9I* rs757343 Polymorphisms in the Mortality of Patients Infected with Different Variants of SARS-CoV-2

Hasan Rahman Shawi Shawi,[a] Enayat Anvari,[b,*] and Abolfazl Fateh[c,d,**]

[a] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[b] *Clinical Research Development Unit, Shahid Mostafa Khomeini Hospital, Ilam University of Medical Science, Ilam, Iran*
[c] *Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran*
[d] *Microbiology Research Center (MRC), Pasteur Institute of Iran, Tehran, Iran*

Received for publication December 21, 2022; accepted March 27, 2023 (ARCMED-D-23-00048).

Background and aim. Low vitamin D levels are associated with the severity of coronavirus disease 19 (COVID-19). Vitamin D receptor gene polymorphisms, such as *Tru9I* rs757343 and *FokI* rs2228570, have been suggested to be potential risk factors for severe COVID-19 outcomes. This study investigated how *Tru9I* rs757343 and *FokI* rs2228570 polymorphisms influenced the mortality rate of COVID-19 in relation to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants.

Methods. The polymerase chain reaction-restriction fragment length polymorphism assay was used to genotype *Tru9I* rs757343 and *FokI* rs2228570 genotypes in 1,734 recovered and 1,450 deceased patients.

Results. Our results demonstrated that the high mortality rate was correlated with *FokI* rs2228570 TT genotype in all three variants but was much higher in the Omicron BA.5 variant than in the Alpha and Delta variants. Furthermore, in patients infected with the Delta variant, *FokI* rs2228570 CT genotype was more highly correlated with the mortality rate compared to other variants. Thus, a high mortality rate was correlated with the *Tru9I* rs757343 AA genotype in the Omicron BA.5 variant, whereas this relationship was not observed in the other two variants. The T-A haplotype was related to COVID-19 mortality in all three variants, but its effect was more pronounced in the Alpha variant. Moreover, the T-G haplotype was significantly associated with all three variants.

Conclusion. Our findings showed that the effects of *Tru9I* rs757343 and *FokI* rs2228570 polymorphisms were related to SARS-CoV-2 variants. However, further studies are still required to validate our findings.

KeyWords: COVID-19, SARS-CoV-2 variants, *Tru9I* rs757343, *FokI* rs2228570.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes the coronavirus disease 2019 (COVID-19) was discovered in China near the end of 2019 and developed into a pandemic in March 2020, posing a significant threat to global public health due to its social and financial costs (1,2). Clinical symptoms of COVID-19 can range from asymptomatic infections to acute respiratory distress syndrome. Differences in symptoms and severity of COVID-19 are partially associated with well-known risk factors, such as male gender, older age, and comorbidities, including heart disease, hypertension, obesity, and diabetes (3,4). However, severe consequences have also occurred in healthy, young patients, suggesting that other potential risk factors, including genetic predisposition, may influence the severity of COVID-19 (5). Host

* Co-corresponding author: Enayat Anvari, Clinical Research Development Unit, Shahid Mostafa Khomeini Hospital, Ilam University of Medical Science, Ilam, Iran. Tel/Fax: (+98) 8432227120.; E-mail: anvari_ph@yahoo.com

** Corresponding author: Abolfazl Fateh, Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran; Phone: (+98) 2164112282; FAX: (+98) 2164112213; E-mail: afateh2@gmail.com

0188-4409/$ - see front matter.
https://doi.org/10.1016/j.arcmed.2023.03.006

Please cite this article as: Shawi *et al.*, Role of FokI rs2228570 and Tru9I rs757343 Polymorphisms in the Mortality of Patients Infected with Different Variants of SARS-CoV-2, Archives of Medical Research, https://doi.org/10.1016/j.arcmed.2023.03.006

genetic factors have been shown to play an important role in susceptibility or resistance to various viral infections (2,6). Considering the central function of host genes in the entry of SARS-CoV-2 into cells and the development of an immune response, it seems that a combination of several genes may be involved in the pathogenesis of COVID-19 (7). Therefore, to date, many studies on the relationship between genetic polymorphisms and the severity of COVID-19 have been performed (8–13).

Vitamin D has been shown to play a key role in many antioxidant, anti-fibrotic, anti-inflammatory, and immunomodulatory mechanisms. As a result, vitamin-D deficiency contributes to several pathogenic conditions, including respiratory infections, cardiovascular disorders, cancer, autoimmune disorders, and diabetes (14). Increasing evidence suggests that vitamin-D deficiency is closely associated with an increased risk of COVID-19 infection and of developing COVID-19-related thrombosis (9,15).

The vitamin-D receptor (VDR) which, after binding to its ligand, is translocated to the nucleus. This connection controls and regulates hundreds of genes (16). *VDR* gene is located on chromosome 12q13. Approximately more than 470 single nucleotide polymorphisms (SNPs) have been found in this gene. Four significant SNPs in the *VDR* gene, including rs1544410, rs731236, rs7975232, and rs2228570, can affect the activity, stability and expression levels of *VDR* gene products, and subsequently change the vitamin-D-VDR signaling axis leading to vitamin-D dysfunction. *VDR* polymorphisms have previously been shown to be associated with some infective agents, such as SARS-CoV-2 (17–19).

The rs2228570 (*FokI*), located at the translation start codon of the *VDR* gene, produces two types of proteins, namely a short (F-VDR) and a longer (f-VDR) variants (20). The F-VDR has a unique role in the rs2228570 polymorphism. It affects immune cells function and is always associated with a more robust immune system (21). Moreover, the *VDR* rs757343 (*Tru9I*) polymorphism is located in intron 8 of the *VDR* gene. The *VDR* rs757343 (*Tru9I*) is associated with expected serum levels of vitamin D in patients from German families with type 1 diabetes (22).

Hitherto, there have been no studies focusing on the correlation between *Tru9I* rs757343 and *FokI* rs2228570 and the outcomes of SARS-CoV-2 variants among Iranian population. This study evaluated whether *Tru9I* rs757343 and *FokI* rs2228570 were related to COVID-19 mortality rate based on SARS-CoV-2 variants.

Materials and Methods

Sample Collection

Patients who were visited at one of the teaching hospitals affiliated to Ilam University of Medical Sciences (Kurdish people) between November 2020 and February 2022 during the three peaks of COVID-19 infection (Alpha, Delta, and Omicron BA.5) were included.

Only 3,184 out of 14,117 patients met the following inclusion criteria: a) give consent before participating in the study, b) have Iranian nationality and the same ethnicity, c) have no history of SARS-CoV-2 infection and have not been vaccinated, d) have positive real-time reverse transcription polymerase chain reaction (rtReal time-PCR) test results and be chosen from a single hospital, and e) have no underlying comorbidities such as kidney disease, liver disease, obesity, diabetes, chronic obstructive pulmonary disease, cancer, heart disease, human immunodeficiency virus (HIV), pregnancy, and/or cystic fibrosis.

The 3,184 participants were divided into two groups: 1,734 recovered COVID-19 patients as the control group and 1,450 age- and sex-matched deceased persons who served as the case group. Since we did not have healthy subjects, we considered the recovered patients as healthy controls and compared the results with those of the deceased.

In accordance with WHO COVID-19 clinical care guidelines, our study included adults with mild and moderate disease who recovered and those with severe or critical COVID-19 infection who died.

Mild ilness was defined as any COVID-19 symptoms (fever, malaise, sore throat, nausea, headache, cough, muscle pain, diarrhea, loss of taste and smell, vomiting) without respiratory distress, dyspnea, abnormal chest imaging, or signs of viral pneumonia or hypoxia. Patients with moderate disease had clinical symptoms of pneumonia (fever, cough, dyspnea, and rapid breathing), but there were no signs of severe pneumonia in these patients, such as a SpO2 lower than 90% on room air.

Clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) were present in patients with severe illness, along with one or more of the following: respiratory rate greater than 30 breaths per minute, severe respiratory distress, or room SpO2 of 90%. Patients with acute respiratory distress syndrome (ARDS), septic shock, sepsis, and other severe illness were defined as those with conditions that normally required life-sustaining interventions, such as invasive or non-invasive mechanical ventilation or vasopressors (23).

All clinical parameters, such as 25-hydroxyvitamin D, real-time PCR cycle threshold (Ct) values, fasting blood glucose (FBS), C-reactive protein (CRP), triglycerides (TG), low-density lipoprotein (LDL), cholesterol, high density lipoprotein (HDL), erythrocyte sedimentation rate (ESR), platelets, white blood cells (WBC), creatinine, uric acid, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were evaluated on admission.

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Tru9I *rs757343 and* FokI *rs.2228570 Genotyping*

Total genomic DNA from all patients was extracted with the High-pure PCR Template Preparation Kit (Roche Diagnostics Deutschland GmbH, Mannheim, Germany), following the manufacturer's instructions. Subsequently, the DNA quality and purity were evaluated by gel electrophoresis and NanoDrop spectrophotometer (Thermo Scientific, USA), respectively.

The SNPs (*Tru9I* rs757343 and *FokI* rs2228570) were genotyped with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The forward and reverse primers for *Tru9I* rs757343 were 5′-CTTTGGAGCCTGAGAGATGG-3′ and 5′-CTCCAGTCCAGGAAAGCATC-3′ (235 bp) and for *FokI* rs2228570 were 5′- CTGGCACTGACTCTGGCTCT-3′ and 5′-TGCTTCTTCTCCCTCCCTTT-3′ (247 bp), respectively.

For *Tru9I* rs757343, the PCR assay was carried out by initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 59°C for 30 s, 72°C for 30 s, and final extension at 72°C for 7 min. The PCR condition for *FokI* rs2228570 was carried out by initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 50 s, and final extension at 72°C for 10 min. The PCR products were then digested using the respective restriction enzymes, which included *Tru9I* and *FokI* enzymes (Fermentas, Vilnius, Lithuania), following the manufacturer's instructions. The digested products were then electrophoresed on a 2.5% agarose gel.

Product sizes were 235 bp for the GG genotype and 162 bp and 73 bp for the AA genotype in *Tru9I* rs757343 and 247 bp for the CC genotype, and 185 bp and 62 bp for the TT genotype in *FokI* rs2228570 (17). At least 10% of the samples were randomly genotyped using the Sanger sequencing technique on an ABI 3500 DX Genetic Analyzer (ABI, Thermo Fisher Scientific, Waltham, MA, USA) to corroborate the PCR-RFLP results. MEGA Version 11.0 was used to evaluate the results (https://www.megasoftware.net/).

Statistical Analyses

The Shapiro-Wilk test was used to determine the normality of the variables. The different variables were expressed as number (No), percentage (%), mean, and standard deviation (SD). The χ^2 test was used to compare qualitative variables between two groups, and the Mann-Whitney *U*-test was used for variables with non-normal distribution. Multivariate logistic regression analysis was used to examine the impact of individual risk factors on the results. Additionally, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A two-sided *p*-value of less than 0.05 was used to determine statistical significance. All statistical calculations were performed with SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA).

The SNPStats online application was used to determine the Hardy-Weinberg equilibrium (HWE), four models of inheritance (dominant, codominant, overdominant, and recessive), and the minor allele frequency (MAF) of the chosen variant. The optimal model was determined using Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) (http://bioinfo.iconcologia.net/SNPStats).

Results

Characteristics of Patients

Table 1 shows a summary of the characteristics of the participants in the COVID-19 research. In total, 3,184 patients participated in the study, of whom 1,022 were Alpha variant with a mean age of 53.0 ± 12.7 years, 1,026 were Delta variant with a mean age of 58.0 ± 11.8 years, and 1,136 were Omicron BA.5 variant, with a mean age of 53.7 ± 12.9 years. The frequency of males and females in the Alpha variant was 525 (51.4%) and 497 (48.6%), in the Delta variant was 546 (53.2%) and 480 (46.8%), and in the Omicron BA.5 variant was 598 (52.6%) and 538 (47.4%), respectively. The mean 25-hydroxy vitamin D ($p = 0.029$) and qPCR Ct values ($p < 0.001$) in the Delta variant were lower than those in the Alpha and Omicron BA.5 variants, which was statistically significant.

Tru9I *rs757343 and* FokI *rs2228570 Polymorphisms and COVID-19 Mortality Adjusted by SARS-CoV-2 Variants*

The COVID-19 mortality rate was significantly higher in patients with the *Tru9I* rs757343 AA and GA genotypes than in those with the GG genotype. Patients with the TT genotype showed a higher COVID-19 mortality rate in the *FokI* rs2228570 polymorphism. Patients who had recovered from COVID-19 also had the *FokI* rs2228570 CC genotype.

Table 2 tabulates the results of the inheritance model analysis of the *Tru9I* rs757343 and *FokI* rs2228570 polymorphisms in patients. The codominant inheritance models (lowest AIC and BIC values) were found to be the best fit for both *Tru9I* rs757343 and *FokI* rs2228570. The *FokI* rs2228570 TT genotype was associated to a high risk of COVID-19 mortality ($p < 0.0001$, OR 4.30, 95% CI 3.49–5.30), and the *Tru9I* rs757343 GA genotype was associated with an increased risk of COVID-19 mortality ($p < 0.0001$, OR 4.31, 95% CI 3.56–5.21).

In both recovered and deceased patients, the *Tru9I* rs757343 and *FokI* rs2228570 polymorphisms were incompatible with HWE ($p < 0.0001$). It should be emphasized that *Tru9I* rs757343 and *FokI* rs2228570 are not in HWE, which may explain their association with the disease. The

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

Variables	SARS-CoV-2 variants			*p*
	Alpha (*n* = 1,022)	**Delta (*n* = 1,026)**	**Omicron BA.5 (*n* = 1,136)**	
Deceased/Improved patients	479/543 (46.9/53.1%)	674/352 (65.7/34.3%)	297/839 (26.1/73.9%)	<0.001*
Mean age ± SD	53.0 ± 12.7	58.0 ± 11.8	53.7 ± 12.9	0.128
Sex (male/female)	525/497 (51.4/48.6%)	546/480 (53.2/46.8%)	598/538 (52.6/47.4%)	0.692
ALT, IU/L (mean ± SD) (Reference range: 5–40)	38.5 ± 24.8	40.8 ± 24.7	35.8 ± 24.2	0.001
AST, IU/L (mean ± SD) (Reference range: 5–40)	34.9 ± 15.5	34.5 ± 14.0	31.9 ± 14.4	<0.001*
ALP, IU/L (mean ± SD) (Reference range: up to 306)	190.2 ± 84.7	188.6 ± 74.0	177.2 ± 83.5	<0.001*
Cholesterol, mg/dL (mean ± SD) (Reference range: 50–200)	116.1 ± 34.1	120.5 ± 40.5	123.1 ± 39.4	<0.001*
TG, mg/dL (mean ± SD) (Reference range: 60–165)	124.1 ± 54.9	121.6 ± 48.8	126.9 ± 55.9	0.245
LDL, mg/dL (mean ± SD) (Reference range: up to 150)	82.8 ± 45.1	85.3 ± 45.3	104.7 ± 48.3	<0.001*
HDL, mg/dL (mean ± SD) (Reference range: >40)	32.5 ± 11.3	32.1 ± 11.5	33.6 ± 11.7	0.039*
WBC, 10^9/L (mean ± SD) (Reference range: 4000–10000)	7627.3 ± 2843.2	7599.2 ± 2715.7	7704.9 ± 2807.7	0.297
CRP, mg/L (mean ± SD) (Reference range: <10 mg/L Negative)	61.6 ± 21.5	63.9 ± 22.0	60.2 ± 21.7	0.122
ESR, mm/1st h (mean ± SD) (Reference range: 0–15)	50.1 ± 16.0	52.3 ± 16.0	49.1 ± 16.1	0.025
FBS, mg/dL (mean ± SD) (Reference range: 70–100)	107.1 ± 41.6	109.8 ± 43.2	106.5 ± 40.7	0.716
Platelets × 1000/cumm (mean ± SD) (Reference range: 140000–400000)	184 ± 71	185 ± 74	184 ± 69	0.994
Uric acid, mg/dL (mean ± SD) (Reference range: 3.6–6.8)	4.8 ± 1.8	4.4 ± 1.7	5.2 ± 1.8	<0.001*
Creatinine, mg/dL (mean ± SD) (Reference range: 0.6–1.4)	0.9 ± 0.3	1.0 ± 0.3	0.8 ± 0.3	<0.001*
qPCR Ct value	20.1 ± 6.4	17.4 ± 6.1	21.9 ± 6.0	<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	0.029*
FokI rs2228570				<0.001*
CC	463 (45.3%)	319 (31.1%)	540 (47.5%)	
CT	318 (31.1%)	421 (41.0%)	436 (10.1%)	
TT	241 (23.6%)	286 (27.9%)	160 (42.4%)	
Tru9I rs757343				<0.001*
GG	360 (35.2%)	620 (60.4%)	982 (86.4%)	
GA	593 (58.0%)	319 (31.1%)	115 (10.1%)	
AA	69 (6.8%)	87 (8.5%)	39 (3.5%)	

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TG: triglyceride; LDL: low density lipoprotein; HDL: high density lipoprotein; WBC: white blood cells; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; FBS: fasting blood glucose; SD: standard deviation; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.
*Statistically significant (<0.05).

MAF for *Tru9I* rs757343 (T) and *FokI* rs2228570 (C) polymorphisms in deceased patients was higher than in those who recovered.

Distributions of FokI *rs2228570 and* Tru9I *rs757343 Polymorphism Among SARS-CoV-2 Variants*

SARS-CoV-2 variants were strongly correlated with mortality. The Delta and Omicron BA.5 respectively were related to high and low death rates (p <0.001). As for the Alpha variant, the frequencies of *FokI* rs2228570 CC, CT, and TT genotypes were 463 (45.3%), 318 (31.1%), and 241 (23.6%), respectively. The frequencies of *FokI* rs2228570 CC, CT, and TT genotypes in the Delta variant were 319 (31.1%), 421 (41.0%), and 286 (27.9%), respectively. Also, that of the Omicron BA.5 variant was 540 (47.5%), 436 (10.1%), and 160 (42.4%), respectively (Table 1).

When we adjusted SARS-CoV-2 variants with *FokI* rs2228570 genotypes, the results showed that a high mortality rate was correlated with *FokI* rs2228570 TT genotype in all three variants and that this amount was much higher in Omicron BA.5 variant (OR 9.56, 95% CI 6.42–14.24) than Alpha (OR 3.43, 95% CI 2.46–4.78) and Delta (OR 3.04, 95% CI 2.16–4.27) variants. Also, *FokI* rs2228570 CT genotype in patients infected with Delta variant (OR

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Table 2. *Tru9I* rs757343 and *FokI* rs2228570 polymorphisms association with COVID-19 mortality adjusted by SARS- CoV-2 variants

***FokI* rs2228570**		**Groups**					
Model	**Genotype**	**Recovered patients**	**Deceased patients**	**OR (95% CI)**	***p***	**AIC**	**BIC**
Allele	C	2428 (70.0%)	1391 (48.0%)	-	-	-	-
	T	1040 (30.0%)	1509 (52.0%)	-	-	-	-
Codominant	C/C	902 (52.0%)	420 (29.0%)	1.00	<0.0001*	3845.8	3876.1
	C/T	624 (36.0%)	551 (38.0%)	1.78 (1.50–2.12)			
	T/T	208 (12.0%)	479 (33.0%)	4.30 (3.49–5.30)			
Dominant	C/C	902 (52.0%)	420 (29.0%)	1.00	<0.0001*	3914.8	3939.0
	C/T-T/T	832 (48.0%)	1030 (71.0%)	2.44 (2.09–2.85)			
Recessive	C/C-C/T	1526 (88.0%)	971 (67.0%)	1.00	<0.0001*	3887.7	3912.0
	T/T	208 (12.0%)	479 (33.0%)	3.25 (2.69–3.94)			
Overdominant	C/C-T/T	1110 (64.0%)	899 (62.0%)	1.00	0.371	4044.9	4069.2
	C/T	624 (36.0%)	551 (38.0%)	1.07 (0.92–1.25)			
Minor allele frequency (T)		0.30	0.52	-	-	-	-
Tru9I rs757343							
Allele	G	2968 (86.0%)	1983 (68.0%)	-	-	-	-
	A	500 (14.0%)	917 (32.0%)	-	-	-	-
Codominant	G/G	1313 (75.7%)	649 (44.8%)	1.00	<0.0001*	3793.7	3824.0
	G/A	342 (19.7%)	685 (47.2%)	4.31 (3.56–5.21)			
	A/A	79 (4.6%)	116 (8.0%)	2.65 (1.92–3.65)			
Dominant	G/G	1313 (75.7%)	649 (44.8%)	1.00	<0.0001*	3799.7	3824.0
	G/A-A/A	421 (24.3%)	801 (55.2%)	3.93 (3.29–4.69)			
Recessive	G/G-G/A	1655 (95.4%)	1334 (92.0%)	1.00	0.011*	4039.2	4063.5
	A/A	79 (4.6%)	116 (8.0%)	1.49 (1.09–2.03)			
Overdominant	G/G-A/A	1392 (80.3%)	765 (52.8%)	1.00	<0.0001*	3828.0	3852.3
	G/A	342 (19.7%)	685 (47.2%)	3.79 (3.15–4.55)			
Minor allele frequency (A)		0.14	0.32	-	-	-	-

COVID-19: coronavirus disease; OR: Odds ratios; CI: confidence intervals; AIC: Akaike information criterion; BIC: Bayesian information criterion; OR: Odds ratios; CI: Confidence intervals
*Statistically significant (<0.05).

3.14, 95% CI 2.31–4.29) compared to other variants was more related to the mortality rate (Table 3).

In the case of the Alpha variant, the frequencies of *Tru9I* rs757343 GG, GA, and AA genotypes were 360 (35.2%), 593 (58.0%), and 69 (6.8%), respectively. The frequencies of *Tru9I* rs757343 GG, GA, and AA genotypes in the Delta variant were 620 (60.4%), 319 (31.1%), and 87 (8.5%), respectively. Also, in the Omicron BA.5 variant were 982 (86.4%), 115 (10.1%), and 39 (3.5%), respectively (Table 1).

When we adjusted SARS-CoV-2 variants with *Tru9I* rs757343 genotypes, the results showed that a high mortality rate was correlated with *Tru9I* rs757343 AA genotype in the Omicron BA.5 variant (OR 2.17, 95% CI 1.13–4.15). At the same time, this relationship was not seen in the other two variants (Table 3).

According to the results, the C-G haplotype was more common in all SARS-CoV-2 variants. The T-A haplotype was related to COVID-19 mortality in all three variants, but its effect was more pronounced in the Alpha variant (OR 12.81, 95%CI 8.73–18.79). Moreover, the T-G haplotype was significantly associated with all three variants (Table 4).

Factors Related to COVID-19 Mortality

The association between risk factors and COVID-19 mortality was examined using a multivariate logistic regression model. The COVID-19 mortality rate was correlated with mean age, ALT, HDL, LDL, FBS, uric acid, creatinine, ESR, 25-hydroxyvitamin D, real-time PCR Ct values, SARS-CoV-2 variants, *FokI* rs2228570, and *Tru9I* rs757343 (Table 5).

Discussion

This comprehensive study examined the frequency of the *FokI* rs2228570 and *Tru9I* rs757343 polymorphisms in COVID-19 patients in relation to SARS-CoV-2 variants and the association between these SNPs and mortality rates in Iranian individuals.

Our findings indicate that an increased risk of COVID-19-related death was associated to the C allele of the *FokI* rs2228570 polymorphism. The MAF (T-allele) for *FokI* rs2228570 in the studied patients was 0.40, and this value was higher in the deceased patients (0.52) than in the recovered ones (0.30). According to the NCBI db-

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Table 3. *Tru9I* rs757343 and *FokI* rs2228570 genotypes association with SARS-CoV-2 variants

Variants	rs2228570 genotypes	Recovered patients	Deceased patients	OR (95% CI)
Alpha	C/C	277	186	1.00
	C/T	193	125	0.96 (0.72–1.29)
	T/T	73	168	3.43 (2.46–4.78)
Delta	C/C	167	152	1.00
	C/T	109	312	3.14 (2.31–4.29)
	T/T	76	210	3.04 (2.16–4.27)
Omicron	C/C	458	82	1.00
BA.5	C/T	322	114	1.98 (1.44–2.72)
	T/T	59	101	9.56 (6.42–14.24)
Variants	**rs757343 genotypes**	**Recovered patients**	**Deceased patients**	**OR (95% CI)**
Alpha	G/G	360	1	1.00
	G/A	151	441	-
	A/A	32	37	-
Delta	G/G	229	391	1.00
	G/A	98	22	1.32 (0.99–1.76)
	A/A	25	17	1.45 (0.89–2.38)
Omicron	G/G	724	258	1.00
BA.5	G/A	93	22	0.66 (0.41–1.08)
	A/A	22	17	2.17 (1.13–4.15)

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; OR: Odds ratios; CI: Confidence intervals

Table 4. SARS-CoV-2 variants and *Tru9I* rs757343 and *FokI* rs2228570 haplotype

Haplotypes	Frequency	Alpha OR (95% CI)	Delta OR (95% CI)	Omicron BA.5 OR (95% CI)
CG	0.5652	1.00	1.00	1.00
TA	0.1881	12.81 (8.73–18.79)	1.36 (1.11–1.67)	1.55 (1.14–2.09)
CA	0.0345	1.16 (0.89–1.36)	0.27 (0.15–1.01)	0.97 (0.83–1.41)
TG	0.2123	4.51 (3.04–6.68)	3.08 (2.34–4.06)	4.45 (3.47–5.70)

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; OR: Odds ratios; CI: Confidence intervals.

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

Factors		
Baseline Predictors	**OR (95 % CI)**	***p*-value**
Mean age ± SD	0.927 (0.909–0.946)	<0.001*
ALT, IU/L	0.981 (0.971–0.957)	<0.001*
HDL, mg/dL	1.032 (1.012–1.051)	0.001*
LDL, mg/dL	1.016 (1.011–1.022)	<0.001*
FBS, mg/dL	0.991 (0.986–0.996)	0.001*
Uric acid, mg/dL	2.095 (1.790–2.451)	<0.001*
Creatinine, mg/dL	0.064 (0.032–0.128)	<0.001*
ESR, (mm/1st h)	0.971 (0.957–0.985)	0.001*
25-hydroxyvitamin D, (ng/Ml)	1.029 (1.009–1.049)	0.004*
Real-time PCR Ct values	2.174 (1.985–2.382)	<0.001*
SARS-CoV-2 variants	1.802 (1.355–2.398)	<0.001*
FokI rs2228570	1.849 (1.280–2.670)	0.001*
Tru9I rs757343	0.190 (0.116–0.312)	<0.001*

ALT: alanine aminotransferase; HDL: high density lipoprotein; LDL: low density lipoprotein; FBS: fasting blood glucose; ESR: erythrocyte sedimentation rate; Ct: cycle threshold; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SD, standard deviation; OR: Odds ratios; CI: Confidence intervals
*Statistically significant (<0.05).

Please cite this article as: Shawi *et al.*, Role of FokI rs2228570 and Tru9I rs757343 Polymorphisms in the Mortality of Patients Infected with Different Variants of SARS-CoV-2, Archives of Medical Research, https://doi.org/10.1016/j.arcmed.2023.03.006

SNP database, MAF in the other regions was identified in Asians (0.438), East Asians (0.431), South Asians (0.250), other Asians (0.456), Africans (0.275), African Americans (0.275), Europeans (0.388), and Latin Americans (0.292) (24).

In this study, the MAF (A-allele) was 0.22 for *Tru9I* rs757343. This value of A-allele, as reported in PubMed, is similar to Asian (0.207), East Asian (0.216), South Asian (0.119), other Asian (0.207), African (0.075), European (0.124), Latin American (0.123), and African American (0.074) (25). The *Tru9I* rs757343 A-allele frequency was lower in the recovered patients (0.14) than in the deceased ones (0.32).

The analysis of genotype data in connection to vitamin D levels demonstrated the role of vitamin D homeostasis and its metabolic pathway in regulating susceptibility to COVID-19 severity. The important role of vitamin D in enhancing host defense against SARS-CoV-2 or other viral infections may be responsible for these genotypic variations in COVID-19 disease outcomes (26,27).

In this study, vitamin D levels in the Delta variant were lower than those in the Alpha and Omicron BA.5 variants, and also the mortality rate was higher than in the other two. Vitamin D increases CD4+ T cells in adaptive immunity and stimulates the production of virus-specific antibodies via activating T lymphocyte-dependent B lymphocytes, especially in epithelial cells (28). It is hypothesized that individuals infected with the Delta variant may have much lower levels of epithelial cells contributing to systemic responses than individuals infected with its predecessors. Reduced epithelial cell contact may also lead to a less adaptive response and decrease the risk of cytokine storm. Despite the lack of evidence about Delta-specific effects on the cytokine storm, the results suggest that it may be less severe in infections caused by Delta than by previous variants. In areas where Delta was more prevalent, there were more reports of intensive care unit (ICU) admissions and respiratory support, suggesting that the lungs may have been more severely affected (29).

The Omicron variant of SARS-CoV-2 replicates more rapidly in the bronchi, but less effectively in the lung parenchyma compared to other SARS-CoV-2 variants (30,31). As a result, the Omicron variant is less likely to induce pneumonia, and the lesions are often mild and difficult to measure accurately (32). All lesions were mild pneumonias, mainly with ground-glass opacities and some small consolidations. Adequate vitamin D levels have been shown to play an important role in lung recovery. It was speculated that vitamin D sufficiency may improve pneumonia lesions by accelerating viral clearance and balancing the inflammatory response (33). It is possible that one of the main reasons for the lower mortality in patients with the Omicron BA.5 variant in our study was the higher vitamin D levels.

Genetic differences may explain the severity of COVID-19 infection among different populations. Genetic analysis should be applied to the design of risk models to improve the effectiveness of surveillance programs. This may lead to better stratification and individualized assessment of the best long-term interventions (34). The association between *VDR* gene polymorphisms and susceptibility to COVID-19 has been investigated in many studies (17,18,35,36).

The results also demonstrated the genetic contribution of two specific *VDR* haplotypes—*FokI* rs2228570 and *Tru9I* rs757343—to COVID-19, highlighting the importance of genotypic changes in defining disease severity in populations. For example, the TT genotype in *FokI* rs2228570 and the AA and GA genotype in *Tru9I* rs757343 were associated to severe COVID-19 outcomes.

The *FokI* rs2228570 polymorphism in exon 2 at the 5′ end of the *VDR* gene is called a start codon polymorphism. When the "T" allele is present, the VDR protein is three amino acids longer, whereas the "C" allele produces a shorter VDR protein linked to a 1.7-fold higher transcriptional activity (37). This polymorphism showed different patterns in different variants in terms of disease severity. In this study, a high mortality rate was correlated with the *FokI* rs2228570 TT genotype in all three variants, and this was much higher in the Omicron BA.5 than in the Alpha and Delta variants. In addition, the *FokI* rs2228570 CT genotype in patients infected with the Delta variant compared to the other variants was more closely related to the mortality rate. Some studies have confirmed these results, but the merit of our research was the investigation of this polymorphism according to different SARS-CoV-2 variants (17,18,35).

The *FokI* rs2228570 T allele impairs the potency of the *VDR* complex to bind to vitamin D-responsive genetic elements (38). The *FokI* rs2228570 TT genotype has been reported to carry a seven-fold increased risk of acute lower respiratory tract infections than the *FokI* rs2228570 CC genotype (39). A meta-analysis study revealed that enveloped viral infections, such as respiratory syncytial virus, were more common in those with the *FokI* rs2228570 T allele (38). Additionally, the *FokI* rs2228570 CC genotype was associated with a better prognosis of liver cirrhosis (40).

In our study, the results showed that the high mortality rate was correlated with the *Tru9I* rs757343 AA genotype in the Omicron BA.5 variant. At the same time, this relationship was not observed in the other two variants. In one study, except in the severe/critical and mild/moderate groups, in which the G and A alleles were characterized as protective and risk factors, respectively, *Tru9I* rs757343 showed no significant variations in allelic distribution between matched group comparisons. The genotypic frequencies of *Tru9I* rs757343 showed no discernible correlation with COVID-19 severity or clinical symptoms (17). This polymorphism is located on the *VDR* gene at the 3′ end

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of intron 8. *Tru9I* rs757343 does not alter the amino acid sequence of the coding protein but may influence gene expression through control of mRNA stability or linkage disequilibrium with other SNPs that influence disease susceptibility (41). It appears that this difference was due to sample size and comparison with different SARS-CoV-2 variants in this study.

The T-A haplotype was related to COVID-19 mortality in all three variants, but its effect was more pronounced in the Alpha variant. Moreover, the T-G haplotype was significantly associated with all three variants in our study. We hypothesized that these two SNPs behave differently in different SARS-CoV-2 variants. However, the mechanism that generates this behavior is unknown.

Previous research focused mainly on the relationship between vitamin D status and COVID-19 disease. This work is one of the limited studies that has investigated the association between these two polymorphisms and different variants of SARS-CoV-2. However, further studies with different SARS-CoV-2 variants are needed to confirm the results of this study.

Although our study has particular strengths, there remain gaps that need to be addressed. In this study, due to the lack of access to healthy individuals, the results were compared between those who recovered and those who died. In addition, the population we studied was from a specific geographical area. Therefore, the results cannot be generalized and further research in different contexts in patients of different ethnicities are needed.

In conclusion, our results indicated that low vitamin D levels were related to COVID-19 mortality, especially in Delta variant cases. Also, it was shown that the *FokI* rs2228570 polymorphism could be associated with COVID-19 mortality in three different SARS-CoV-2 variants, whereas the *Tru9I* rs75734 polymorphism only influenced mortality in patients infected with the Omicron BA.5 variant.

Conflict of Interests

The authors declare that there are no conflicts of interest.

Acknowledgment

We would like to thank all the patients who participated in the study.

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