IMMUNOREGULATION

2025. Vol 8

Research Paper

Association of Transmembrane Immunoglobulin and Mucin Domain rs10515746 Polymorphism With Severity and Mortality of COVID-19 in the Iranian Population

Ensie Sadat Mirsharif[1], Abdolrahman Rostamian[2], Mohammadreza Salehi[3], Nayere Askari[1], Tooba Ghazanfari[1*]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran.
2. Rheumatology Research Center, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Infectious and Tropical Medicines, Tehran University of Medical Sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Mirsharif ES, Rostamian A, Salehi M, Askari N, Ghazanfari T. Association of Transmembrane Immunoglobulin and Mucin Domain rs10515746 Polymorphism With Severity and Mortality of COVID-19 in the Iranian Population. Immunoregulation. 2025; 8:E17. http://dx.doi.org/10.32598/Immunoregulation.8.17

http://dx.doi.org/10.32598/Immunoregulation.8.17

Article info:
Received: 10 Apr 2025
Accepted: 27 May 2025
Available Online: 13 Jun 2025

Keywords:
T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), Polymorphism, rs10515746, COVID-19

ABSTRACT

Background: T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) is an inhibitory immune checkpoint regulating T cell immune responses. This study aimed to evaluate the association between the TIM-3 rs10515746 single-nucleotide polymorphism and the severity and mortality of coronavirus disease 2019 (COVID-19) among Iranians.

Materials and Methods: Genomic DNA was extracted from peripheral blood nucleated cells of 828 COVID-19 patients and 166 healthy controls without COVID-19. The *TIM-3* polymorphic site was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. To determine whether genetic variation was associated with hepatitis A virus cellular receptor 2 (*HAVCR-2*) messenger ribonucleic acid (mRNA) expression, total RNA was extracted from 175 COVID-19 patients and 65 healthy controls, and *HAVCR-2* expression levels were quantified using reverse transcription-polymerase chain reaction (RT-PCR).

Results: The genotypic frequencies of TIM-3 574A>C (rs10515746) polymorphism were in accordance with the Hardy-Weinberg equilibrium in the healthy control subjects (P>0.05). No significant difference was observed in their distribution frequencies of rs10515746 between the genotypes and alleles among COVID-19 patients vs. controls, inpatients vs outpatients, intensive care unit (ICU) vs non-ICU admitted patients, and survivors vs. non-survivors under different genetic models. Also, no significant difference was observed in the mRNA expression of *HAVCR-2* in COVID-19 patients with other genotypes.

Conclusion: COVID-19 infection was not associated with the TIM-3 574A>C (rs10515746) polymorphism among the Iranians.

*** Corresponding Author:**
Tooba Ghazanfari, Professor.
Address: *Immunoregulation Research Center, Shahed University, Tehran, Iran.*
Phone: *+98 (21) 66418216*
E-mail: *tghazanfari@yahoo.com, ghazanfari@shahed.ac.ir*

Introduction

The third coronavirus epidemic in the last decade, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, poses a fundamental threat to global health, economy, and society. According to the World Health Organization (WHO), as of April 2024, there were over 775 million COVID-19 patients and over 7 million deaths worldwide. The complex interaction between SARS-CoV-2 and the host immune system activates various inflammatory pathways, leading to excessive inflammation and a cytokine storm [1]. The immune system plays a vital role in the development of COVID-19, and the severity of the disease may correlate with the dysregulation of inflammatory immune responses [1-3].

The human transmembrane immunoglobulin and mucin domain (*TIM*) gene family comprises *TIM-1, TIM-3,* and *TIM-4*, located on chromosome 5q33.2. These cell-surface glycoproteins have specific structural elements, including the transmembrane region, mucin domain, immunoglobulin domain, signal peptide, and intracellular tail containing a phosphorylation site. T cell receptors and co-stimulatory signals [4], along with TIM proteins, control the growth and effector functions of Th1/Th2 cells [4]. Despite the critical role of the *TIM* gene family products in immune responses, there is limited knowledge regarding *TIM* regulation in disease development [5, 6].

TIM-3 is an immune checkpoint molecule that regulates innate and adaptive immune responses [7, 8]. This immunoregulatory protein is the first specific surface molecule to identify human and mouse Th1 cells, binds to its ligands, galectin-9 and phosphatidylserine, and inhibits T-cell activation and proliferation. Also, TIM-3 promotes T-cell exhaustion and apoptosis, leading to immune dysfunction [7]. Th17 cells may also express lower levels of TIM-3 than Th1 cells [9]. Other immune cells, such as CD8+ T cells, regulatory T cells, monocytes, natural killer cells, dendritic cells, and mast cells, may also express *TIM-3* [6, 10]. Many viral infections show a positive correlation among TIM-3 levels, viral load, and disease progression, thereby supporting the proposed inhibitory role of this protein [11–13]. Due to its essential role in regulating the host's immune response, excessive or suppressed inflammatory responses resulting from dysregulation of *TIM-3* expression may lead to an autoimmune disorder or viral evasion; however, an exact mechanism is unclear [10, 14].

The human hepatitis A virus cellular receptor 2 (*HAVCR-2*) gene encodes TIM-3, a member of the immunoglobulin superfamily [15]. Previous investigations have reported that polymorphisms in *HAVCR-2* are associated with some cancers, allergic diseases, and autoimmunity [16, 17]. The most prevalent type of genetic variation in the human population is single-nucleotide polymorphisms (SNPs) [7]. Previous association studies have identified specific genetic variations, known as SNPs, associated with the mortality rate of COVID-19 in Iranian patients. These SNPs include the CC genotypes of angiotensin-converting enzyme 2 (ACE2) rs2285666, TT genotypes of ACE2 rs2074192, CC genotypes of interferon-induced transmembrane protein 3 (IFITM3) rs12252, T allele of IFITM3 rs34481144, G allele of IFITM3 rs6598045, AA genotypes of ABO rs657152, CC genotypes of transmembrane serine protease 2 rs12329760, and tripartite motif containing 22 variants (rs7113258 TT, rs1063303 GG, and rs7935565 GG) [18-27].

Since COVID-19 is primarily caused by a dysregulated immune response and inflammatory cytokine storm, genetic variations in genes involved in T cell-mediated immunity may affect COVID-19 severity and mortality [15]. The rs10515746 polymorphism, with a MAF>0.05 and located in the promoter region of the *HAVCR-2* gene, has been the subject of most investigations. Allelic polymorphic variation from rs10515746 (C/A) disrupts Th1/Th2 cell differentiation and causes macrophage activation dysregulation [6].

Study objectives or hypotheses

Given the adverse co-stimulatory effect of TIM-3 on T-mediated immune responses and the significant impact of TIM-3 574A>C (rs10515746) on susceptibility to certain diseases, this study proposes that this genetic variation may also be associated with the development, progression, or outcome of COVID-19. Accordingly, in this study, we examined the genotype and allele frequencies of rs10515746 and *HAVCR-2* mRNA expression in an Iranian COVID-19 population.

Materials and Methods

In the present cross-sectional study, we recruited 828 Iranian COVID-19 patients between January and June 2020. The COVID-19 infection was confirmed by a positive SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) test, a pulmonary infiltrate on a chest x-ray (limited to inpatients), and a clinical assessment by an infectious disease specialist. The exclusion criteria were

exposure to other infectious diseases, experimental sample loss, and non-Iranian patients. Based on the disease severity, according to the WHO interim guidance and comprehensive national guidelines for the diagnosis and treatment of COVID-19, sixth edition [28], the patients were further classified as non-intensive unit care (ICU) admitted (mild: n=297; moderate: n=406) vs ICU admitted (severe: n=44; critical: n=81), and outpatients (mild: n=297) vs inpatients (moderate: n=406; severe: n=44; critical: n=81) groups. Because sampling occurred during the initial peak of SARS-CoV-2 infection in Iran, none of the patients had previously contracted the virus or received vaccinations. The control group included 166 healthy, ethnically matched adults with no underlying diseases or a history of SARS-CoV-2 infection who tested negative for COVID-19 using RT_PCR. Informed consent was obtained from all participants. This study was approved by the Research Ethics Committee of Shahed University, Tehran, Iran.

Genomic DNA extraction and rs10515746 genotyping

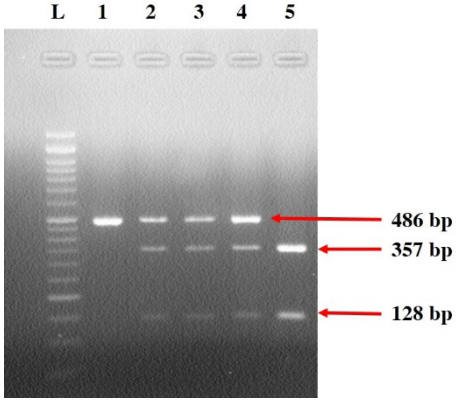
Genomic DNA was extracted from 3 to 5 mL of peripheral blood collected in tubes pretreated with ethylenediaminetetraacetic acid (EDTA) from all participants using the GeneAll® Exgene™ Kit (Korea) according to the manufacturer's instructions and then stored at -20 ˚C.

The PCR-restriction fragment length polymorphism method was used to determine the genotypes of rs10515746 located in the promoter region of *HAVCR-2*. The desired DNA sequence was amplified using forward (5`-TCACTCAAATCAGTCCCTTCATC-3`) and reverse (5' TGGCTGGAACTCAACACTTTC-3') primers to produce a 486 bp amplified DNA fragment of *HAVCR-2*. A reaction mixture of 20 µL was prepared, containing master mix (TEMPase Hot Start 2x Master Mix A, AMPLIQON) (10 µL), primers (10 pM), and genomic DNA (0.02-0.04 µg). The mixture was then subjected to amplification using a PCR thermocycler (Bio-Rad, USA) under the following conditions: 15 min of initial denaturation at 95 ˚C, followed by 35 cycles of denaturation at 95 ˚C for 30 s, annealing at 55 ˚C for 30 s, extension at 72 ˚C for 60 s, and final extension at 72 ˚C for 5 minutes.

BCC-I, an allele-specific restriction enzyme (New England Biolabs, USA), was used to digest the PCR product (486 bp) to identify the polymorphic site. Briefly, 10 µL of the PCR product was mixed with 0.5 µL of BCC-I restriction enzyme, 2.5 µL of its 10X buffer, and 12 µL of nuclease-free water. The mixture was incubated at 37 ˚C for 16 h. Subsequently, the reaction was made inactive for 20 min at 65 ˚C. After electrophoresis on a 2% agarose gel, the digested fragments were visually examined under an ultraviolet transilluminator/gel documentation system. The A allele fragment remained intact, while the C allele was fragmented into 128 and 357 bp pieces (Figure 1). Meanwhile, 10% of the amplified DNA samples were sequenced to validate the accuracy of the results. The results were completely consistent.

RNA extraction and *HAVCR-2* expression

The association between the rs10515746 polymorphism and *HAVCR-2* mRNA expression in 175 confirmed COVID-19 patients (mild = 61, moderate = 61, severe=17, critical=36) and 65 healthy controls without underlying diseases was investigated using real-time PCR. The patients and controls belonged to the same ethnic group. A stratified sampling technique was used to select the samples because there were few patients in the higher-severity subgroups. The GeneAll® Hybrid-RTM



IMMUNOREGULATION

Figure 1. TIM-3 rs10515746 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping

Note: Lane L: DNA ladder (50 bp); Lane No. 1 is genotype AA (486 bp), Lane No. 2 is genotype AC (486 bp, 357 bp, and 128 bp), Lane No. 5 is genotype CC (357 bp and 128 bp).

Kit (Cat. No. 305-101, Korea) was utilized to extract total RNA from 3 mL of peripheral venous blood in tubes that had been pretreated with ethylenediaminetetraacetic acid (EDTA) according to the manufacturer's guidelines. A spectrophotometer (Nanodrop, Thermo Scientific, USA) was used to measure the concentration and purity of the isolated RNA. Next, a bioanalyzer (ABI, USA) was used to evaluate the sample's integrity. According to the manufacturer's guidelines, 1000 nanograms (ng) of RNA was converted to complementary DNA (cDNA) using the high-capacity cDNA reverse transcription Kit (ABI, USA). Subsequently, they were kept at -20 ˚C. Real-time PCR reactions were conducted in a 20 µL mixture containing 10 µL of SYBR Green real-time PCR master mix (AMPLIQON), 2 µL of cDNA diluted at a ratio of 1:10, 5 pM of each primer (forward primer: 5′- TTCCAAGGATGCTTACCA -3′, and reverse primer: 5′- CCTGCTCCGATGTAGATG -3′), and 6 µL of nuclease-free water. It was performed on a StepOne™ real-time PCR system (ThermoFisher Scientific, USA).

The best thermal cycling conditions were as follows: A 16-minute initial denaturation at 95 ˚C, followed by 40 cycles of denaturation at 95 ˚C for 30 s, annealing at 61 ˚C for 1 minute, and extension at 72 ˚C for 30 s. All samples were tested thriplicate. Human β-actin mRNA expression was used as a reference gene for internal controls.

Statistical analysis

The allele and genotype frequencies of rs10515746 among the study groups were calculated using the Pearson chi-square test in SPSS software, version 26.0 (SPSS Inc., Chicago, IL, USA) and SNPStats. The relationship between rs10515746 genotypes and the severity and mortality of COVID-19 was reported using odds ratios and 95% confidence intervals (CI). In addition, Hardy-Weinberg equilibrium deviations in the study groups were evaluated using the online SNPStats software [29]. The Mann-Whitney U test was used to compare *HAVCR-2* expression across rs10515746 genotypes. Statistical significance was defined as a P<0.05. Diagrams were created using GraphPad Prism software, version 8.0.1.

Results

Subject characteristics

Table 1 presents the population characteristics of patients with COVID-19 and controls. No significant differences were observed in gender or body mass index between the groups (P>0.05). However, the mean age was significantly higher in COVID-19 patients than in controls, inpatients than outpatients, ICU-admitted patients than non-ICU-admitted patients, and non-survivors than survivors (P≤0.001).

The most common clinical manifestations (Table 2) of COVID-19 patients were fever (56.4%), dyspnea (54.4%), weakness (47.9%), dry cough (47.5%), myalgia (41.9%), chills (33.1%), anorexia (31.4%), headache (26.2%), diarrhea (17.3%), and anosmia (14.7%). Regarding disease severity, the frequency of dyspnea (P≤0.001), headache (P=0.005), myalgia (P=0.038), anosmia (P=0.021), and diarrhea (P=0.011) was higher

Table 1. Demographic characteristics of COVID-19 patients

Variables		Mean±SD/No. (%)		P	Mean±SD/No. (%)		P
		COVID-19 Patients (n=828)	Healthy Control (n=166)		Inpatients (n=531)	Outpatients (n=297)	
Age		53±17	46±14	≤0.001	57±16	44±14	≤0.001
BMI (kg/m²)		26.49±4.59	26.29±4.37	0.64	26.71±5	26.14±3.83	0.11
Gender	Female	328(39.6)	62(37.3)	0.59	205(38.6)	123(41.4)	0.43
	Male	500 (60.4)	104(62.7)		326(61.4)	174(58.6)	

Variables		Mean±SD/No. (%)		P	Mean±SD/No. (%)		P
		ICU Admitted Patients (n=125)	Non-ICU Admitted Patients (n=703)		Survivor Patients (n=742)	Non-survivor Patients (n=86)	
Age		61±17	51±16	≤0.001	51±16	65±14	≤0.001
BMI (kg/m²)		27.28±5.7	26.35±4.35	0.13	26.44±4.51	26.9±5.26	0.44
Gender	Female	47(37.6)	281(40)	0.62	301(40.6)	27(31.4)	0.1
	Male	78(62.4)	422(60)		441(59.4)	59(68.6)	

*P<0.05 significant.

Table 2. Frequency of underlying disease and clinical manifestation in COVID-19 patients

Variables		%
Underlying disease	Hypertension	29.7
	Diabetes mellitus	24.3
	Heart diseases	17.1
	Respiratory diseases	8.2
	Renal diseases	7.3
	Thyroid disease	4.2
	Immune diseases	2.9
	Cerebrovascular accident	2.6
	Cancer	2.5
	Liver diseases	1.8
Clinical manifestation	Fever	56.4
	Dyspnea	54.4
	Weakness	47.9
	Dry cough	47.5
	Myalgia	41.9
	Chill	33.1
	Anorexia	31.4
	Headache	26.2
	Diarrhea	17.3
	Vomiting	16.4
	Anosmia	14.7
	Pharyngitis	11.8
	Chest pain	11.5
	Productive cough	6.5
	Abdominal pain	6.1
	Nausea	6.1
	Rhinorrhea	4.6
	Vertigo	3.1

IMMUNOREGULATION

in ICU-admitted patients than in non-ICU-admitted patients. The most common underlying diseases (Table 2) among COVID-19 patients were hypertension (29.7%), diabetes (24.3%), and heart disease (17.1%).

Association of T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) 574A>C (rs10515746) polymorphism with COVID-19

The genotype frequencies of the TIM-3 574A>C (rs10515746) polymorphism were in Hardy-Weinberg equilibrium in healthy control subjects ($P > 0.05$). In controls, the minor allele frequency (MAF) of rs10515746 was 0.16, which is consistent with the 1000 Genomes (0.15) and HapMap (0.19) projects [30]. No significant differences were observed in the genotype and allele frequency distributions of TIM-3 574A>C (rs10515746) between COVID-19 patients and control subjects, or among COVID-19 subgroups, under different genetic models (Table 3, Supplementary Table 1). Subsequent analysis using logistic regression adjusted for age, gender, and comorbidities, showed no significant differences between our study groups across the different inheritance models. As previously mentioned, a significant difference was observed in the mean age among the study groups. Further analysis, after dividing the age range into < 40, 40–80, and >80 years, revealed that age variation did not confound the results.

Table 3. The frequency of the rs10515746 genotypes among COVID-19 patients' subgroups under hereditary models' analysis

Genotypes		No. (%)		P[b]	OR (95% CI)
		COVID-19 Patients (n=828)	Healthy Control (n=166)		
Codominant model	CC	583(70.4)	118(71.1)	0.58	1
	AC	212(25.6)	44(26.5)		1.32 (0.77-2.27)[a]
	AA	33(4)	4(2.4)		
Dominant model	CC	583(70.4)	118(71.1)	0.86	1
	AC+AA	245(29.6)	48(28.9)		0.78 (0.42-1.46)[a]
Recessive model	CC+AC	795(96)	162(97.6)	0.3	1
	AA	33(4)	4(2.4)		2.90 (0.37-22.55)[a]
Overdominant	AA+CC	616(74.4)	122(73.5)	0.81	1
	AC	212(25.6)	44(26.5)		1.11 (0.59-2.10)[a]
Allele frequency	C	1378(83)	280(84)	0.62	1
	A	278(17)	52(16)		1.08 (0.79-1.48)

Genotypes		No. (%)		P[b]	OR (95% CI)
		Inpatients (n=531)	Outpatients (n=297)		
Codominant model	CC	378(71.2)	205(69)	0.79	1
	AC	133(25.1)	79(26.6)		1.08 (0.80, 1.46)[a]
	AA	20(3.8)	13(4.4)		
Dominant model	CC	378(71.2)	205(69)	0.51	1
	AC+AA	153(28.8)	92(31)		0.91 (0.63, 1.32)[a]
Recessive model	CC+AC	511(96.2)	284(95.6)	0.67	1
	AA	20(3.8)	13(4.4)		1.11 (0.5, 2.48)[a]
Overdominant	AA+CC	398(75)	218(73.4)	0.62	1
	AC	133(25.1)	79(26.6)		1.08 (0.73, 1.61)[a]
Allele frequency	C	889(84)	489(82)	0.49	1
	A	173(16)	105(18)		1.10 (0.85, 1.41)

Genotypes		No. (%)		P[b]	OR (95% CI)
		Non-ICU Patients (n=703)	ICU Admitted Patients (n=125)		
Codominant model	CC	91(72.8)	492(70)	0.55	1
	AC	31(24.8)	181(25.8)		1.12 (0.76, 1.65)[a]
	AA	3(2.4)	30(4.3)		
Dominant model	CC	91(72.8)	492(70)	0.52	1
	AC+AA	34(27.2)	211(30)		0.92 (0.58, 1.46)[a]
Recessive model	CC+AC	122(97.6)	673(95.7)	0.32	1
	AA	3(2.4)	30(4.3)		1.61 (0.46,5.55)[a]
Overdominant	AA+CC	94(75.2)	522(74.2)	0.82	1
	AC	31(24.8)	181(25.8)		1 (0.63, 1.63)[a]
Allele frequency	C	213(0.85)	1165(83)	0.37	1
	A	37(15)	241(17)		1.18 (0.82, 1.69)

Genotypes		No. (%)		P[b]	OR (95% CI)
		Survivor Patients (n=742)	Non-survivor Patients (n=86)		
Codominant model	CC	520(70.1)	63(73.3)	Unreliable	1
	AC	189(25.5)	23(26.7)		0.79 (0.49,1.28)[a]
	AA	33(4.5)	0		

Genotypes		No. (%)		P[b]	OR (95% CI)
		Survivor Patients (n=742)	Non-survivor Patients (n=86)		
Dominant model	CC	520(70.1)	63(73.3)	0.54	1
	AC+AA	222(29.9)	23(26.7)		1.10 (0.64, 1.92)[a]
Recessive model	CC+AC	709(95.5)	86(100)	Unreliable	1
	AA	33(4.5)	0		-
Overdominant	AA+CC	553(74.5)	63(73.3)	0.8	1
	AC	189(25.5)	23(26.7)		1.15(0.66, 2.02)[a]
Allele frequency	C	1229(83)	149(87)	0.21	1
	A	255(17)	23(13)		0.76 (0.49, 1.18)

Abbreviations: ICU: Intensive care unit; ORs: Odds ratio; CI: Confidence interval. IMMUNOREGULATION

[a]Logistic regression analyses were used for calculating odds ratios, adjusted for age, gender, and comorbidities, [b]P was calculated by χ^2 test; It should be noted that statistical analysis was not feasible for cases labeled as 'unreliable' due to the absence of the AA genotype among non-survivor COVID-19 patients.

Association of TIM-3 574A>C (rs10515746) polymorphism with *HAVCR-2* expression in patients with COVID-19

The *HAVCR-2* expression in COVID-19 patients was markedly decreased compared to healthy controls. To explore the impact of the TIM-3 574A>C (rs10515746) polymorphism, located in the promoter region of *HAVCR-2*, on *HAVCR-2* mRNA expression, mRNA levels of *HAVCR-2* were evaluated in patients with different rs10515746 genotypes. According to the data presented in Table 4, based on disease severity and mortality, no significant differences in *HAVCR-2* mRNA levels were observed among patients with different genotypes across all COVID-19 subgroups (P>0.05).

Discussion

In this study, we investigated the association between the TIM-3 rs10515746 polymorphism and COVID-19 risk and clinical outcomes. To the best of our knowledge, this study is the first to assess the role of the rs10515746 polymorphism in TIM-3 in COVID-19 severity and mortality. No significant alterations were found in the genotype and allele frequency distributions of TIM-3 574A>C (rs10515746) between patients with COVID-19 and controls or between COVID-19 subgroups. Our results suggested that this polymorphism may not affect the risk and progression of COVID-19 among Iranians. To the best of our knowledge, no study has examined the association between this polymorphism and COVID-19 or other viral diseases. Several studies have investigated the association between rs10515746 and susceptibility to respiratory disorders and autoimmune diseases. The results of the meta-analysis showed that in the dominant genetic model, TIM-3 rs10515746 was significantly correlated with an increased risk of asthma development in Asians [31]. Sadri et al. reported a substantial correlation between asthma susceptibility and the TIM-3 574G>T polymorphism [32]. Additionally, based on literature reviews, there was no significant association between TIM-3 rs10515746 and autoimmune conditions, such as Behçet disease [33], rheumatoid arthritis [34], systemic lupus erythematosus [6], and type 1 diabetes [5].

Furthermore, based on the location of rs10515746 (-574A>C) in the *HAVCR-2* promoter region, we evaluated the association between *HAVCR-2* mRNA levels and COVID-19 severity and mortality by genotype distribution. Although our results showed that *HAVCR-2* mRNA levels in COVID-19 patients were significantly lower than those in healthy participants, no significant differences in *HAVCR-2* mRNA expression were observed among the COVID-19 subgroups with different genotypes. Accordingly, our results suggest that rs10515746 may not be a functional polymorphism that influences *HAVCR-2* mRNA levels.

Similarly, we found that in the same COVID-19 patients, SNPs in other immune checkpoint genes, such as *CTLA-4* rs231775 and *PD-1* rs10204525, were not associated with mRNA levels of *CTLA-4* and *PDCD-1* [19, 35]. To date, no study has examined the effect of the rs10515746 polymorphism on *HAVCR-2* mRNA expression in COVID-19 patients. Moreover, there are conflicting reports on the relationship between the rs10515746 polymorphism and the transcriptional activity of the *HAVCR-2* gene. Zhang et al. demonstrated no significant relationship between rs10515746 and *HAVCR-2* mRNA expression [36]. In contrast, another study reported significantly lower *HAVCR-2* mRNA expression in monocytes, CD4+ T cells, and CD8+ T cells in individuals with the rs10515746 CA genotype compared to those with the CC genotype [37].

Table 4. *HAVCR-2* mRNA levels in COVID-19 patient subgroups according to rs10515746 genotypes

	Variables	AA		AC		CC		P[1]	P[2]	P[3]
		No.	Mean±SD (Median)	No.	Mean±SD (Median)	No.	Mean±SD (Median)			
HAVCR-2 mRNA expression	Healthy control	0	-	21	0.98±0.48 (0.91)	52	1.33±0.87 (1.16)	-	-	0.123
	All patients	8	1.26±0.53 (1.06)	47	1.02±0.82 (0.79)	133	1.15±0.94 (0.82)	0.09	0.149	0.403
COVID-19 patients	Hospitalized patients (inpatients)	4	1.27±0.41 (1.26)	32	1.08±0.96 (0.75)	88	1.22±1.09 (0.81)	0.208	0.275	0.478
	Non-hospitalized patients (outpatients)	4	1.25±0.7 (0.96)	15	0.91±0.34 (0.93)	45	1.03±0.51 (0.83)	0.453	0.342	0.746
	Non-survivors	0	-	10	0.51±0.29 (0.45)	23	1.04±1.15 (0.62)	-	-	0.136
	Survivors	8	1.26±0.53 (1.06)	37	1.16±0.86 (0.93)	110	1.18±0.89 (0.86)	0.278	0.218	0.952
	Non-ICU admitted patients	8	1.26±0.53 (1.06)	31	1.28±0.88 (1.01)	91	1.25±0.94 (0.98)	0.614	0.383	0.764
	ICU admitted patients	0	-	16	0.52±0.27 (0.52)	42	0.94±0.92 (0.68)	-	-	0.072

IMMUNOREGULATION

[1]Comparison between AA and AC genotypes, [2]Comparison between AA and CC genotypes, [3]Comparison between AC and CC genotypes.

TIM-3 plays a vital role in modulating the immune system's reaction. The TIM3/Gal-9 axis primarily regulates the inflammatory process triggered by interferon (IFN)-producing CD4+ and CD8+ T cells. Previous studies have reported increased TIM-3 expression on CD4+ and CD8+ T cells in COVID-19 patients [38-40]. Some studies have reported a correlation between COVID-19 severity and sTIM-3 levels. Chavez-Galan et al. report a significant elevation in the levels of soluble programmed cell death-ligand 1 (sPD-L1), soluble TIM-3 (sTIM-3), and matrix metalloproteinase 7 (sMMP-7) in severe COVID-19 patients [41]. Reanalyzing the single-cell RNA sequencing datasets of COVID-19 patients revealed a decrease in natural killer T (NKT) cells and an increase in TIM-3 +NKT cells, which correlated with disease severity and mortality [42]. Although previous research has shown that TIM-3 expression on immune cells is significantly higher in COVID-19 patients, our results showed no significant differences in *HAVCR-2* mRNA expression between COVID-19 subgroups stratified by rs10515746 genotypes. Therefore, further research on other genetic variants that may be associated with changes in *HAVCR-2* expression in patients with COVID-19 is needed.

Conclusion

In conclusion, we demonstrated that COVID-19 is not associated with the TIM-3 574A>C (rs10515746) genotype and allele frequencies in the Iranian population. However, larger-scale studies across different ethnic groups are required to validate our findings. Furthermore, regarding TIM-3's role as a negative immunoregulator in regulating inflammatory immune responses, it is also suggested to investigate additional TIM-3 SNPs and to explore the gene's function and precise mechanisms in patients with COVID-19.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Shahed University, Tehran, Iran (Code: IR.SHAHED.REC.1400.046).

Funding

This research was financially supported by Shahed University and the Ministry of Health and Medical Education of Iran.

Authors' contributions

Conceptualization and study design: Ensie Sadat Mirsharif and Tooba Ghazanfari; Data collection: Ensie Sadat Mirsharif, Tooba Ghazanfari, Abdolrahman Rostamian, and Mohammadreza Salehi; Data analysis and interpretation: Ensie Sadat Mirsharif, Nayere Askari, and Tooba Ghazanfari; Writing and final approval: All authors.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgements

The authors extend their special gratitude to all the participants and medical staff who provided diagnostic and treatment services for patients in Iran.

References

[1] Tan LY, Komarasamy TV, Rmt Balasubramaniam V. Hyperinflammatory immune response and COVID-19: A double edged sword. Frontiers in Immunology. 2021; 12:742941. [DOI:10.3389/fimmu.2021.742941] [PMID]

[2] Tahaghoghi-Hajghorbani S, Zafari P, Masoumi E, Rajabinejad M, Jafari-Shakib R, Hasani B, et al. The role of dysregulated immune responses in COVID-19 pathogenesis. Virus Research. 2020; 290:198197. [DOI:10.1016/j.virusres.2020.198197] [PMID]

[3] Tahaghoghi-Hajghorbani S, Zafari P, Masoumi E, Rajabinejad M, Jafari-Shakib R, Hasani B, et al. The role of dysregulated immune responses in COVID-19 pathogenesis. Virus Research. 2020; 290:198197. [DOI:10.1002/cbin.11517] [PMID]

[4] Mariat C, Sánchez-Fueyo A, Alexopoulos SP, Kenny J, Strom TB, Zheng XX. Regulation of T cell dependent immune responses by TIM family members. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2005; 360(1461):1681-5. [DOI:10.1098/rstb.2005.1706] [PMID]

[5] Brück P, Ramos-Lopez E, Bartsch W, Böhme A, Badenhoop K. TIM-3 polymorphisms in type 1 diabetes families. Journal of Human Genetics. 2008; 53(6):559-64. [DOI:10.1007/s10038-008-0286-y] [PMID]

[6] Li WX, Chen GM, Yuan H, Yao YS, Li RJ, Pan HF, et al. Polymorphisms of the TIM-1 and TIM-3 genes are not associated with systemic lupus erythematosus in a Chinese population. Mutagenesis. 2011; 26(4):507-11. [DOI:10.1093/mutage/ger009] [PMID]

[7] Wang S, Zhang X, Leng S, Xu Q, Sheng Z, Zhang Y, et al. Immune checkpoint-related gene polymorphisms are associated with primary immune thrombocytopenia. Frontiers in Immunology. 2021; 11:615941.[DOI:10.3389/fimmu.2020.615941] [PMID]

[8] Liu F, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. Journal of Hematology & Oncology. 2018; 11(1):126. [DOI:10.1186/s13045-018-0667-4] [PMID]

[9] Anderson AC, Anderson DE. TIM-3 in autoimmunity. Current Opinion in Immunology. 2006; 18(6):665-9. [DOI:10.1016/j.coi.2006.09.009] [PMID]

[10] Han G, Chen G, Shen B, Li Y. Tim-3: An activation marker and activation limiter of innate immune cells. Frontiers in Immunology. 2013; 4:449. [DOI:10.3389/fimmu.2013.00449] [PMID]

[11] Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. The Journal of Experimental Medicine. 2008; 205(12):2763-79. [DOI:10.1084/jem.20081398] [PMID]

[12] McMahan RH, Golden-Mason L, Nishimura MI, McMahon BJ, Kemper M, Allen TM, et al. Tim-3 expression on PD-1+ HCV-specific human CTLs is associated with viral persistence, and its blockade restores hepatocyte-directed in vitro cytotoxicity. The Journal of Clinical Investigation. 2010; 120(12):4546-57. [DOI:10.1172/JCI43127] [PMID]

[13] Wu W, Shi Y, Li J, Chen F, Chen Z, Zheng M. Tim-3 expression on peripheral T cell subsets correlates with disease progression in hepatitis B infection. Virology Journal. 2011; 8:113. [DOI:10.1186/1743-422X-8-113] [PMID]

[14] Tang F, Wang F, An L, Wang X. Upregulation of tim-3 on CD4+ T cells is associated with Th1/Th2 imbalance in patients with allergic asthma. International Journal of Clinical and Experimental Medicine. 2015; 8(3):3809. [Link]

[15] Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein TIM-3 regulates macrophage activation and severity of an autoimmune disease. Nature. 2002; 415(6871):536-41. [DOI:10.1038/415536a] [PMID]

[16] Lee J, Phong B, Egloff AM, Kane LP. TIM polymorphisms--genetics and function. Genes and Immunity. 2011; 12(8):595-604. [DOI:10.1038/gene.2011.75] [PMID]

[17] Bai J, Li X, Tong D, Shi W, Song H, Li Q. T-cell immunoglobulin- and mucin-domain-containing molecule 3 gene polymorphisms and prognosis of non-small-cell lung cancer. Tumour Biology. 2013; 34(2):805-9. [DOI:10.1007/s13277-012-0610-1] [PMID]

[18] Mohammadi NG, Namaki S, Hashemi SM, Salehi M, Ghaffarpour S, Ghazanfari T. Impact of the MCP-1-2518A>G polymorphism on COVID-19 severity in the Iranian population: A case-control study. International Immunopharmacology. 2023; 119:110217. [DOI:10.1016/j.intimp.2023.110217] [PMID]

[19] Mirsharif ES, Rostamian A, Salehi M, Askari N, Ghazanfari T. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) +49A>G (rs231775) gene polymorphism is not associated with COVID-19 severity and mortality in an Iranian population. Heliyon. 2023; 10(1):e23308. [DOI:10.1016/j.heliyon.2023.e23308] [PMID]

[20] Khalilzadeh F, Sakhaee F, Sotoodehnejadnematalahi F, Zamani MS, Ahmadi I, Anvari E, et al. Angiotensin-converting enzyme 2 rs2285666 polymorphism and clinical parameters as the determinants of COVID-19 severity in Iranian population. International Journal of Immunogenetics. 2022; 49(5):325-332. [DOI:10.1111/iji.12598] [PMID]

[21] Ahmadi I, Afifipour A, Sakhaee F, Zamani MS, Mirzaei Gheinari F, et al. Impact of interferon-induced transmembrane protein 3 gene rs12252 polymorphism on COVID-19 mortality. Cytokine. 2022; 157:155957. [DOI:10.1016/j.cyto.2022.155957] [PMID]

[22] Gholami M, Sakhaee F, Mirzaei Gheinari F, Sotoodehnejadnematalahi F, Ghazanfari Jajin M, Zamani MS, et al. Interferon-induced transmembrane protein 3 rs34481144 C/T genotype and clinical parameters related to progression of COVID-19. Journal of Immunology Research. 2023; 2023:2345062. [DOI:10.1155/2023/2345062] [PMID]

[23] Mirzaei Gheinari F, Sakhaee F, Gholami M, Sotoodehnejadnematalahi F, Zamani MS, Ahmadi I, et al. ABO rs657152 and blood groups are as predictor factors of COVID-19 mortality in the Iranian population. Disease Markers. 2022; 2022:5988976. [DOI:10.1155/2022/5988976] [PMID]

[24] Beheshti Shirazi SS, Sakhaee F, Sotoodehnejadnematalahi F, Zamani MS, Ahmadi I, Anvari E, et al. rs12329760 polymorphism in transmembrane serine protease 2 gene and risk of coronavirus disease 2019 mortality. BioMed Research International. 2022; 2022:7841969. [DOI:10.1155/2022/7841969] [PMID]

[25] Raheem Juhi Al-Kaabi N, Khameneh SC, Montazeri M, Mardasi M, Amroabadi JM, et al. On the relationship between tripartite motif-containing 22 single-nucleotide polymorphisms and COVID-19 infection severity. Human Genomics. 2022; 16(1):33. [DOI:10.1186/s40246-022-00394-z] [PMID]

[26] Gholami M, Sakhaee F, Sotoodehnejadnematalahi F, Zamani MS, Ahmadi I, Anvari E, et al. Increased risk of COVID-19 mortality rate in IFITM3 rs6598045 G allele carriers infected by SARS-CoV-2 delta variant. Human Genomics. 2022; 16(1):60. [DOI:10.1186/s40246-022-00434-8] [PMID]

[27] Sheikhian F, Sadeghi Mofrad S, Tarashi S, Ghazanfari Jajin M, Sakhaee F, Ahmadi I, et al. The impact of ACE2 polymorphisms (rs1978124, rs2285666, and rs2074192) and ACE1 rs1799752 in the mortality rate of COVID-19 in different SARS-CoV-2 variants. Human Genomics. 2023; 17(1):54. [DOI:10.1186/s40246-023-00501-8] [PMID]

[28] World Health Organization (WHO). Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: Interim guidance, 28 January 2020. Geneva: World Health Organization; 2020. [Link]

[29] Institut Català d'Oncologia. SNPStats: A web tool for the analysis of association studies. L'Hospitalet de Llobregat: 2026. [Link]

[30] No author. HapMap Project [Internet]. 2026 [Updated 2026 March 4. Available from [Link]

[31] Wei W, Huang J, Ma Y, Hao C, Zhang S. Association between gene polymorphisms of T cell immunoglobulin domain and mucin domain-3 and risk of asthma: A systematic review and meta-analysis. Iranian Journal of Allergy, Asthma, and Immunology. 2021; 20(1):1-10. [DOI:10.18502/ijaai.v20i1.5407] [PMID]

[32] Sadri M, Ganjalikhani-Hakemi M, Akbari P, Salehi R, Rastaghi S, Ghasemi R, et al. Association between+ 4259 T> G and-574 G> T polymorphisms of TIM-3 with asthma in an Iranian Population. Iranian Journal of Allergy, Asthma and Immunology. 2017; 321-8. [Link]

[33] Ataei M, Behfarjam F, Jadali Z. TIM-3 genetic variants and risk of Behçet disease in the Iranian population. Anais Brasileiros de Dermatologia. 2019; 94(4):429-33. [DOI:10.1590/abd1806-4841.20198022] [PMID]

[34] Razi B, Reykandeh SE, Alizadeh S, Amirzargar A, Saghazadeh A, Rezaei N. TIM family gene polymorphism and susceptibility to rheumatoid arthritis: Systematic review and meta-analysis. Plos One. 2019; 14(2):e0211146.[DOI:10.1371/journal.pone.0211146] [PMID]

[35] Mirsharif ES, Rostamian A, Salehi M, Askari N, Ghazanfari T. Association of programmed cell death 1 (PD-1) gene polymorphism (rs10204525) with COVID-19 severity and mortality: A case-control study in the Iranian population. International Immunopharmacology. 2023; 119:110114. [DOI:10.1016/j.intimp.2023.110114] [PMID]

[36] Zhang J, Daley D, Akhabir L, Stefanowicz D, Chan-Yeung M, Becker AB, et al. Lack of association of TIM3 polymorphisms and allergic phenotypes. BMC Medical Genetics. 2009; 10:62. [DOI:10.1186/1471-2350-10-62] [PMID]

[37] Wang M, Ji B, Wang J, Cheng X, Zhou Q, Zhou J, et al. Tim-3 polymorphism downregulates gene expression and is involved in the susceptibility to ankylosing spondylitis. DNA and Cell Biology. 2014; 33(10):723-8. [DOI:10.1089/dna.2014.2456] [PMID]

[38] Shahbazi M, Moulana Z, Sepidarkish M, Bagherzadeh M, Rezanejad M, Mirzakhani M, et al. Pronounce expression of Tim-3 and CD39 but not PD1 defines CD8 T cells in critical Covid-19 patients. Microbial Pathogenesis. 2021; 153:104779. [DOI:10.1016/j.micpath.2021.104779] [PMID]

[39] Modabber Z, Shahbazi M, Akbari R, Bagherzadeh M, Firouzjahi A, Mohammadnia-Afrouzi M. TIM-3 as a potential exhaustion marker in CD4+ T cells of COVID-19 patients. Immunity, Inflammation and Disease. 2021; 9(4):1707-15. [DOI:10.1002/iid3.526] [PMID]

[40] Martín-Quirós A, Maroun-Eid C, Avendaño-Ortiz J, Lozano-Rodríguez R, Valentín Quiroga J, Terrón V, et al. Potential role of the galectin-9/TIM-3 axis in the disparate progression of SARS-CoV-2 in a married couple: A case report. Biomedicine Hub. 2021; 6(1):48-58. [DOI:10.1159/000514727] [PMID]

[41] Chavez-Galan L, Ruiz A, Martinez-Espinosa K, Aguilar-Duran H, Torres M, Falfan-Valencia R, et al. Circulating levels of PD-L1, TIM-3 and MMP-7 are promising biomarkers to differentiate COVID-19 patients that require invasive mechanical ventilation. Biomolecules. 2022; 12(3):445. [DOI:10.3390/biom12030445] [PMID]

[42] Yang J, Chang T, Tang L, Deng H, Chen D, Luo J, et al. Increased expression of tim-3 is associated with depletion of NKT cells in SARS-CoV-2 infection. Frontiers in Immunology. 2022; 13:796682. [DOI:10.3389/fimmu.2022.796682] [PMID]

Supplementary Table 1. The Frequency of the rs10515746 Genotypes Among COVID-19 Patients With Different Severity Under Hereditary Models' Analysis

Genotypes		Severity										
		Mild	Moderate	Severe	Critical	P	P1	P2	P3	P4	P5	P6
		No. (%)										
Codominant model,	CC	205(69)	287(70.7)	34(77.3)	57(70.4)	0.75	0.89	0.47	0.33	0.58	0.31	0.26
	AC	79(26.6)	102(25.1)	8(18.2)	23(28.4)							
	AA	13(4.4)	17(4.2)	2(4.5)	1(1.2)							
Dominant model	CC	205(69)	287(70.7)	34(77.3)	57(70.4)	0.73	0.63	0.25	0.82	0.35	0.95	0.4
	AC+AA	92(31)	119(29.3)	10(22.7)	24(29.6)							
Recessive model	CC+AC	284(95.6)	389(95.8)	42(95.5)	80(98.8)	0.61	0.9	0.96	0.14	0.91	0.15	0.26
	AA	13(4.4)	17(4.2)	2(4.5)	1(1.2)							
Overdominant model	AA+CC	218(73.4)	304(74.9)	36(81.8)	58(71.6)	0.61	0.66	0.22	0.75	0.29	0.54	0.2
	AC	79(26.6)	102(25.1)	8(18.2)	23(28.4)							
Allele frequency	C	489(82)	676(83)	76(86)	137(85)		0.66	0.36	0.51	0.47	0.69	0.7
	A	105(18)	136(17)	12(14)	25(15)							

Data are presented as No. (%).

P: Comparison of the frequency distribution of genotypes and alleles among COVID-19 patients with different severity.

P 1: Comparison of the frequency distribution of genotypes and alleles between mild and moderate COVID-19 patients.

P 2: Comparison of the frequency distribution of genotypes and alleles between mild and severe COVID-19 patients.

P 3: Comparison of the frequency distribution of genotypes and alleles between mild and critical COVID-19 patients.

P 4: Comparison of the frequency distribution of genotypes and alleles between moderate and severe COVID-19 patients.

P 5: Comparison of the frequency distribution of genotypes and alleles between moderate and critical COVID-19 patients.

P 6: Comparison of the frequency distribution of genotypes and alleles between severe and critical COVID-19 patients.