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HLA Class I Genotypes and Their Role in COVID-19 Severity: A Study in the Isfahan Province

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ABSTRACT

Background: The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses a significant global health threat. The host immune response determines the disease severity, with factors like human leukocyte antigen (HLA) genes, age, sex, and nutritional status influencing outcomes. HLA genes, known for their genetic diversity, are implicated in determining susceptibility and severity of infectious diseases. This study investigated the association between HLA class I genotypes and COVID-19 severity in the Isfahan population, Iran.
Materials & Methods: Blood samples were collected from 34 COVID-19 patients with varying levels of disease severity (severe, moderate, and mild). HLA genotyping was performed using polymerase chain reaction-sequence specific primers (PCR-SSP), and in silico analysis assessed the affinity of viral peptides to HLA alleles.
Findings: Statistical analyses revealed that HLA-C07 was more prevalent in patients with severe COVID-19, suggesting a potential association between this allele and the disease severity. Furthermore, HLA-A01 was more prevalent among severe cases, while HLA-A02 and HLA-A03 were less frequent, indicating a possible predisposing role for HLA-A01 and protective roles for HLA-A02 and HLA-A*03.
Conclusion: These findings highlight the role of HLA molecules in COVID-19 severity and offer insights into genetic factors influencing outcomes. Understanding the association of specific HLA alleles, such as HLA-C07, HLA-A01, HLA-A02, and HLA-A03, with the disease progression lays a foundation for advancing personalized preventive and therapeutic approaches. These results contribute to knowledge on host genetics in infectious diseases, paving the way for further research and therapeutic strategies.

Keywords: Corona virus, COVID-19, HLA class I, HLA typing, SARS-CoV-2, Viral peptide

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Introduction

The coronavirus disease 2019 (COVID-19) is a serious and highly contagious respiratory disease caused by a novel RNA virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus was first identified in Wuhan, China, in December 2019. Since then, SARS-CoV-2 has swiftly spread across the globe, triggering the COVID-19 pandemic. As of March 15, 2022, the World Health Organization's COVID-19 dashboard reported over 458 million cases and a staggering death toll surpassing 6 million worldwide [1, 2].

The development of COVID-19 and its clinical manifestations are dependent on the interaction between SARS-CoV-2 and the host immune system. The immune response is influenced by human leukocyte antigen (HLA) genes, age, sex, nutritional status, and physical status [3]. In the immune response to SARS-CoV-2 infection, it is observed that innate immunity acts first by detecting the virus through recognition of pathogen-associated molecular patterns (PAMPs). This is followed by the activation of the adaptive immune system, which helps lower the viral load by engaging T cells and producing antibodies [4]. Both innate and adaptive immunity are vital in mounting effective antiviral defenses, which include the production of various pro-inflammatory cytokines and the activation of CD4+ and CD8+ T cells.

HLA class I molecules, encoded by the highly polymorphic region 6p21 of the human genome, play a pivotal role in orchestrating the immune response to viral infections. These molecules bind to intracellularly processed viral peptides and present them on the cell surface to CD8+ T cells, which then target and destroy infected cells. In addition to this role, HLA class I molecules interact with inhibitory and activating receptors on natural killer (NK) cells, regulating their ability to recognize and eliminate virus-infected or aberrant cells [5, 6]. The ability of specific HLA alleles to influence the function of CD8+ T cells and NK cells highlights their critical importance in determining the efficacy of immune responses and potentially the clinical outcomes of SARS-CoV-2 infection [4-9].

Extensive research has focused on their potential role as a genetic factor influencing the progression of viral infections and clinical outcomes [10]. A large body of evidence indicates a genetic association between HLA class I (A, B, and C) and class II (DRB1, DQA1, and DQB1) alleles and the severity of infectious diseases [11-13], including human immunodeficiency virus (HIV), hepatitis virus, tuberculosis, and malaria [14-21]. For example, in HIV-1 infection, HLA-A*02:05 may reduce the risk of seroconversion, and in the disease caused by SARS-CoV in 2003, increased severity was shown among individuals with HLA-B*46:01 [10, 12, 22]. HLA class I molecules play a vital role in initiating the immune response to COVID-19 infection. After SARS-CoV-2 enters a cell, it triggers the production of its proteins. Some of these proteins are then processed into 8-12 amino acid peptides within the proteasomes of the infected cell. These peptides bind to HLA class I molecules, and the HLA class I-peptide complex is transported to the cell surface, where it interacts with the T cell receptor (TCR) of CD8+ T lymphocytes. This interaction is crucial for initiating a specific immune response against the virus [23].

The connection between HLA class I genotype and susceptibility to SARS-CoV-2 infection has been explored in several studies. Research has identified specific HLA alleles, including HLA-B*07:03, HLA-B*46:01, and HLA-C*08:01, as potential risk factors for severe COVID-19. Conversely, the HLA-C*15:02 allele has been linked to milder cases of infection in

the studied populations [22, 24, 25]. Given the polymorphic nature of HLA genes and the varying distribution of HLA alleles across different populations and ethnic groups, it is essential to conduct similar studies in diverse populations to identify potentially protective or predisposing HLA alleles.

On the other hand, the amino acid sequence and the structure of the virus could play a significant role in its affinity for specific types of HLA alleles and its presentation. Coronaviruses are large enveloped viruses, and their lipid bilayer envelope contains several proteins with different tasks. Spike or S glycoprotein (SP) is a large type 1 transmembrane protein with two domains, S1 and S2, which are responsible for invasion, attachment, and entry into human cells. The receptor-binding domain (RBD) in S1 interacts with angiotensin-converting enzyme 2 (ACE2) on the human host cell surface, and the S2 domain is responsible for virus-cell membrane fusion and high-affinity viral entry [10, 11]. However, how these factors affect the affinity of SARS-CoV-2 epitopes for certain types of HLA alleles in various populations is still unclear.

Given the critical role of HLA molecules in antigen presentation and immune modulation, investigating their association with disease severity could provide valuable insights into the underlying mechanisms of immune susceptibility.

Objectives: This study aimed to explore the relationship between HLA class I genotypes and the severity of COVID-19, contributing to a better understanding of the genetic factors influencing the disease outcomes in the Isfahan population.

Materials and Methods

Subjects: In the current study, blood samples were obtained from 34 patients diagnosed with SARS-CoV-2 (COVID-19) and categorized based on the severity of their clinical manifestations: severe (n=10), moderate (n=15), and mild (n=9). These patients were diagnosed at Al-Zahra and Amin University Hospitals as well as at Nobel Medical Diagnostic Laboratory in Isfahan, Iran, between September 2021 and February 2022 when the predominant circulating variant of SARS-CoV-2 was Delta. The main inclusion criterion was a positive real-time reverse transcriptase polymerase chain reaction (RT-PCR) test of the oropharyngeal swab or endotracheal sample. Patients with a history of chronic disorders, including autoimmune diseases, cancer, diabetes, or hypertension, were excluded from the study. Ethical aspects of this study were approved by the Research Ethics Committee of Isfahan University of Medical Science (IR.ARI.MUI.REC.1400.116), and the study was conducted in accordance with the Declaration of Helsinki [26]. Patients were informed about the study objectives and signed written consent.

Patients were diagnosed based on the Iranian National Committee COVID-19 criteria. Patients with confirmed COVID-19 tests, who were older than 70 years and showed at least one of the following manifestations were enrolled in the severe group: dyspnea, respiratory rate ≥30/min, oxygen saturation ≤93%, more than 50% lung involvement on imaging, respiratory failure shock, or multi-organ damage. The moderate group consisted of outpatients with confirmed COVID-19, who did not meet the severe criteria mentioned above but had mild pneumonia. The mild group included patients who tested positive for COVID-19 but had no clinical respiratory manifestations.

HLA typing: Genomic DNA was extracted from anticoagulated peripheral blood samples using the DNall Plus Blood Genomic DNA Extraction Kit (Rojetechnology Company). The concentration and purity

of the extracted DNA were assessed using a NanoDrop spectrophotometer. A polymerase chain reaction with sequence-specific primers (PCR-SSP) for HLA-ABC was then carried out using a commercial Morgan HLA SSP ABC Typing Kit (TBG, Medigen Biotechnology, Taipei, Taiwan) following the manufacturer's instructions. The resulting PCR products were visualized through electrophoresis on a 2% agarose gel. The gel images were analyzed using tables provided by the manufacturer.

In silico analysis: FASTA format of the spike protein of SARS-CoV-2 was obtained from Uniprot (accession number: P0DTC2). Using the Immune Epitope Database (IEDB), MHC I affinity prediction for different HLA alleles of all lengths was performed by netMHCpan v4.1 method as previously described [8]. Output was sorted based on the predicted score.

Statistical analysis: Calculation and comparison of allele frequencies were done using Chi-square (v2) and Fisher's exact tests with a significance level of $p< .05$ by univariate regression test using IBM SPSS® version 26.

Findings

Table 1 provides demographic and clinical details of the study participants. A total of 34 patients participated in the study, consisting of 19 women and 15 men. The average age of patients in the mild group was 45 years with a standard deviation of 5.2 years. In contrast, the mean age of the moderate and severe groups was 53 years (± 4.9) and 69 years (± 7.4), respectively. The analysis indicated that HLA-A24 and HLA-A02 were the most frequently observed HLA-A alleles across all groups (Graph 1-1). Additionally, HLA-B35 and HLA-C04 emerged as the predominant HLA-B and HLA-C alleles, respectively (Graphs 1-2 and 1-3). Statistical analyses showed a significantly higher frequency of HLA-C07 in patients with severe COVID-19 compared to the mild and moderate groups ($p\leq .05$), suggesting its potential role in the disease progression.

The predicted HLA-peptide binding affinities indicated that HLA-A02 had the highest number of epitopes with strong binding affinity (IC50 < 50 nM) in all groups (Table 3). HLA-B35 was associated with 16 and 17 high-affinity epitopes in the moderate and severe groups, respectively. In contrast, HLA-B14 and HLA-B52, although present in the mild and severe groups, did not show any high-affinity epitope binding. Among HLA-C alleles, fewer peptides were predicted to bind with strong affinity compared to HLA-A and HLA-B alleles.

Table 1) Demographic and clinical data of three groups of patients

	Group 1 (n=9)	Group 2 (n=15)	Group 3 (n=10)
Age (Mean SD)	45	53	69
Gender			
Female	4 (44%)	9 (60%)	6 (60%)
Male	5 (56%)	6 (40%)	4 (40%)
Cardiovascular disease			
Hypertension	1 (11%)	3 (20%)	7 (70%)
Coronary artery disease	-	1 (6.6%)	2 (20%)
Stroke	-	-	1 (10%)
Metabolite disease			
Diabetes	-	2 (13%)	6 (60%)
Obesity	-	4 (26%)	4 (40%)
Chronic respiratory Disease			
Asthma	-	2 (13%)	3 (30%)
Chronic obstructive pulmonary disease	-	1 (6.6%)	2 (20%)

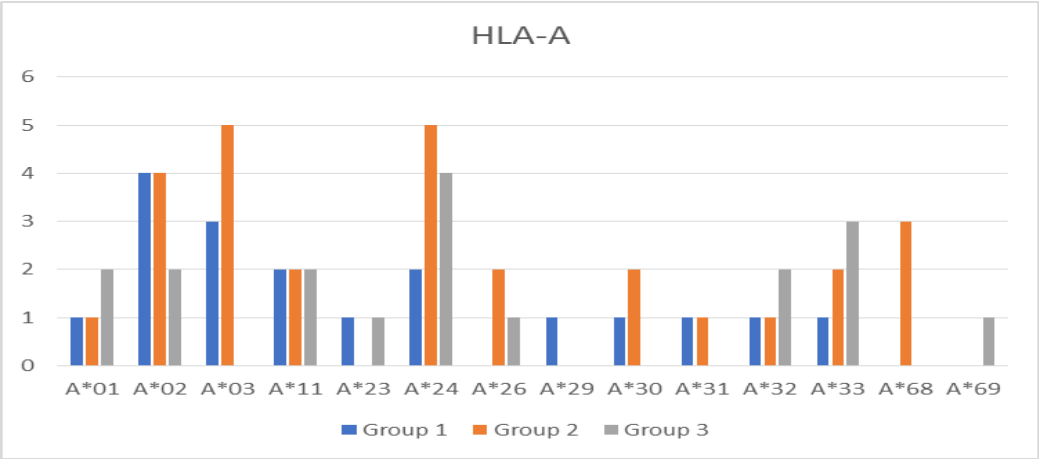

Figure 1) Frequency of HLA-A in the three groups of patients

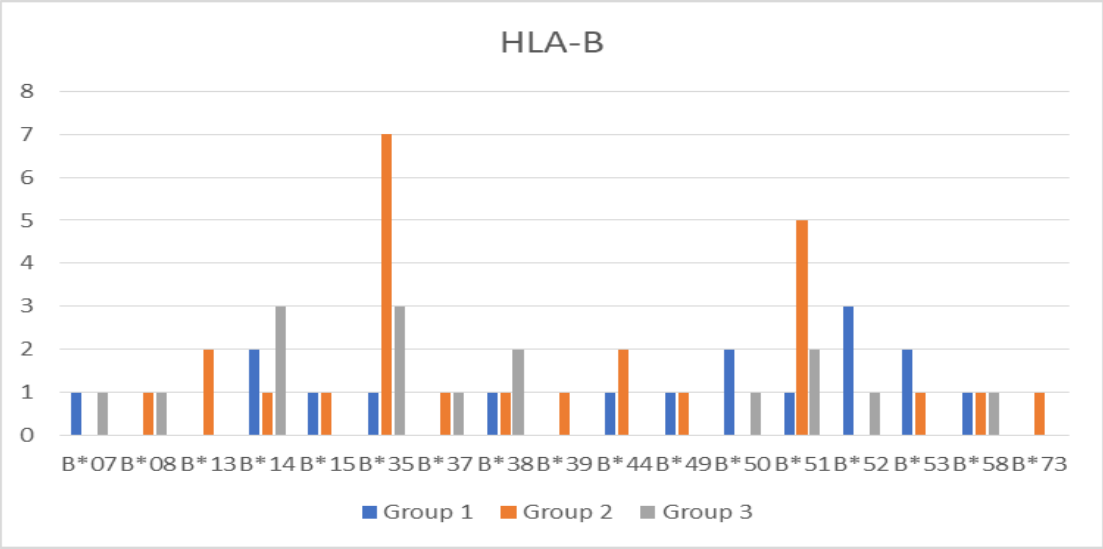

Figure 2) Frequency of HLA-B in the three groups of patients

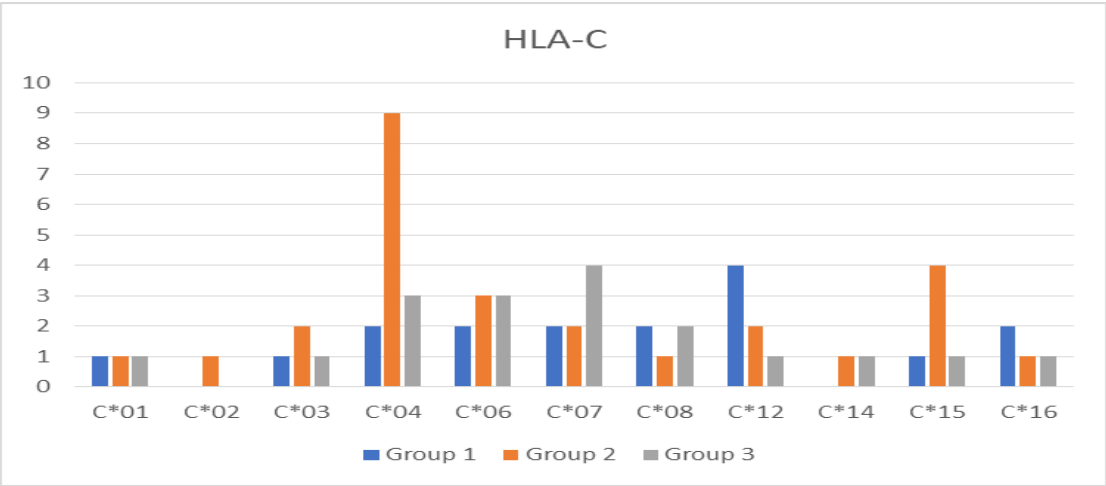

Figure 3) Frequency of HLA-C in the three groups of patients

Table 2) Results of univariate analyses including frequencies, proportions, estimated odds ratios, and *p* values

HLA	HLA alleles	Group	Frequency (mild: n=9, Moderate: n=16, severe:n=10) N (%)	*P*-Value	OR[b] (CI 95%)[c]
HLA-A	A*01	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	1(3.1)	0.23	0.55(0.03-9.33)
		Severe	3(15)	0.106	3.00(0.03-9.33)
	A*02	Mild	4(22.2)	1(reference)	1(reference)
		Moderate	4(12.5)	0.70	0.50(0.11-2.30)
		Severe	2(10)	0.52	0.39(0.06-2.44)
	A*03	Mild	3(16.6)	1(reference)	1(reference)
		Moderate	4(12.5)	0.17	1.15(0.25-5.30)
		Severe	0	NS	NS
	A*11	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	2(6.25)	0.34	0.53(0.07-4.15)
		Severe	3(15)	0.38	1.41(0.21-9.58)
	A*23	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	1(5)	0.50	0.89(0.05-15.44)
	A*24	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	6(18.75)	0.514	2.24(0.41-12.16)
		Severe	4(20)	0.85	2.00(0.32-12.51)
	A*26	Mild	0	1(reference)	1(reference)
		Moderate	2(6.25)	NS	NS
		Severe	1(5)	NS	NS
	A*29	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	0	NS	NS
	A*30	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.46	1.13(0.96-13.44)
		Severe	0	NS	NS
	A*31	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.46	1.13(0.96-13.44)
		Severe	0	NS	NS
	A*32	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	1(3.1)	0.39	0.55(0.03-9.33)
		Severe	2(10)	0.33	1.89(0.16-22.79)
	A*33	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.52	1.13(0.96-13.44)
		Severe	3(15)	0.22	3.00(0.28-31.80)
	A*68	Mild	0	1(reference)	1(reference)
		Moderate	3(10.66)	NS	NS
		Severe	0	NS	NS
	A*69	Mild	0	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	1(5)	NS	NS

HLA	HLA alleles	Group	Frequency (mild: n=9, Moderate: n=16, severe:n=10) N (%)	*P*-Value	OR[b] (CI 95%)[c]
HLA-B	B*07	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	1(5)	0.496	0.89(0.05-15.44)
	B*08	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	1(5)	NS	NS
	B*13	Mild	0	1(reference)	1(reference)
		Moderate	2(6.25)	NS	NS
		Severe	0	NS	NS
	B*14	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	1(3.1)	0.13	0.26(0.02-3.07)
		Severe	3(15)	0.22	1.41(0.21-9.58)
	B*15	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.90	0.55(0.03-9.33)
		Severe	0	NS	NS
	B*35	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	10(31.25)	0.19	4.76(.054-42.28)
		Severe	3(15)	0.92	3.00(0.28-31.80)
	B*37	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	1(5)	NS	NS
	B*38	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	1(3.1)	0.39	0.55(0.03-9.33)
		Severe	2(10)	0.33	1.89(0.16-22.79)
	B*39	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	0	NS	NS
	B*44	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.46	1.13(0.01-13.44)
		Severe	0	NS	NS
	B*49	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	1(5)	0.50	0.89(0.05-15.44)
	B*50	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	1(5)	0.85	0.42(0.03-5.08)
	B*51	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	6(18.75)	0.31	3.15(0.35-29.31)
		Severe	1(5)	0.81	1.89(0.16-22.79)
	B*52	Mild	3(16.6)	1(reference)	1(reference)
		Moderate	0	NS	NS
		Severe	2(10)	0.87	0.26(0.02-2.79)
	B*53	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	1(3.1)	0.66	0.26(0.02-3.07)
		Severe	0	NS	NS
	B*55	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	1(3.1)	0.66	0.55(0.03-9.33)
		Severe	1(5)	0.85	0.42(0.03-5.08)
	B*58	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	1(3.1)	0.66	0.55(0.03-9.33)
		Severe	1(5)	0.85	0.42(0.03-5.08)
	B*73	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	0	NS	NS

HLA	HLA alleles	Group	Frequency (mild: n=9, Moderate: n=16, severe:n=10) N (%)	*P*-Value	OR[b] (CI 95%)[c]
HLA-C	C*01	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.86	1.13(0.01-13.44)
		Severe	1(5)	0.87	0.89(0.05-15.44)
	C*02	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	0	NS	NS
	C*03	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	2(6.25)	0.86	1.13(0.01-13.44)
		Severe	1(5)	0.871	0.89(0.05-15.44)
	C*04	Mild	3(16.6)	1(reference)	1(reference)
		Moderate	12(37.5)	0.07	3.00(0.72-12.55)
		Severe	4(20)	0.395	1.25(0.24-6.54)
	C*06	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	3(9.3)	0.62	0.83(0.12-5.48)
		Severe	2(10)	0.553	1.41(0.21-9.58)
	C*07	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	2(6.25)	0.13	0.53(0.07-4.15)
		Severe	5(25)	0.05	2.67(0.45-15.89)
	C*08	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	1(3.1)	0.23	0.26(0.02-3.07)
		Severe	2(10)	0.56	0.89(0.11-7.06)
	C*12	Mild	4(22.2)	1(reference)	1(reference)
		Moderate	2(6.25)	0.34	0.23(0.04-1.43)
		Severe	1(5)	0.38	0.18(0.02-1.83)
	C*14	Mild	0	1(reference)	1(reference)
		Moderate	1(3.1)	NS	NS
		Severe	1(5)	NS	NS
	C*15	Mild	1(5.5)	1(reference)	1(reference)
		Moderate	5(15.6)	0.15	3.15(0.34-29.31)
		Severe	1(5)	0.38	0.89(0.05-5.44)
	C*16	Mild	2(11.11)	1(reference)	1(reference)
		Moderate	1(3.1)	0.39	0.26(0.02-3.07)
		Severe	1(5)	0.87	0.42(0.03-5.08)

Discussion

Based on previous reports, there are correlations between different specific HLA alleles and susceptibility to some infectious disorders [22, 23, 27]. Some studies have attempted to address this issue in COVID-19 patients in different populations using different genotyping methods [22-24]. There are some reports on the frequency of HLA class I (-A, -B, -C) in COVID-19 patients. To the best of our knowledge, this is the first study to report the frequency of HLA class I in three different groups of patients with mild, moderate, and severe COVID-19 in the Iranian population. Based on the results of low-resolution HLA-typing, patients with severe COVID-19 had a higher frequency of HLA-C*07 compared to the mild and moderate groups, which is consistent with the results of a study by Wang et al. (2020) in China, reporting a higher frequency of HLA-C*07:29 in infected patients rather than in healthy populations [28]. Although these findings should be interpreted with caution due to statistical limitations, our data align with and support previous research. In addition, in the present study, there was a homozygous person in terms of HLA-C*07 among patients with severe disease, while homozygosity for HLA-C*07 was not observed among patients in the mild and moderate groups. Another study in Italy and Spain showed no significant relation between HLA molecules and COVID-19

Table 3) Summary of recognized HLA class I with the highest frequency matched with IEDB output

	Group	HLA Allele with the Highest Frequency	Number of Epitopes That Could Be Recognized Based on Epitope Mapping in IEDB Database with High Affinity (IC50<50 nM)
HLA-A	Group A	A*02	14
		A*03	16
		A*024	7
	Group B	A*02	43
		A*03	16
		A*024	7
		A*11	26
	Group C	A*02	13
		A*33	9
		A*24	7
HLA-B	Group A	B*52	0
		B*14	0
	Group B	B*35	16
		B*51	0
	Group C	B*14	0
		B*35	17
HLA-C	Group A	C*12	3
	Group B	C*04	0
		C*15	2
	Group C	C*07	0
		C*04	0
		C*06	1

infection [29]. However, in another study on the Iranian population, HLA-B*38 frequency was higher in patients than in the uninfected group [30].

The association between highly expressed HLA-A alleles and COVID-19 severity was reported by Shkurnikov et al. (2021) in Russia. They observed that the presence of HLA-A*01 in people increased the risk of COVID-19 severity, while the occurrence of HLA-A*02 and HLA-A*03 was consistent with a low risk of the disease [31].

In the present study, an increase in the incidence of HLA-A*01 was observed among patients with severe disease, which is consistent with the above-mentioned study. Meanwhile, a reduction in the frequency of HLA-A*02 and HLA-A*03 alleles was observed in patients with severe clinical manifestations in comparison with the other two groups (mild and moderate). This is also in line with the above study and the study by Toyoshima et al. (2020), which examined the association between mortality rates and HLA gene variants [32]. The observed differences were not statistically significant, likely due to the limited sample size in this study. Notably, the severe disease group exhibited increased expression of HLA-A*01, with one individual being homozygous for this allele, a scenario not observed in mild or moderate patients. These results suggest a potential predisposition associated with HLA-C*07 and HLA-A*01, while indicating a protective effect of HLA-A*02 and HLA-A*03 alleles in the context of COVID-19 infection. The role of HLA types in presenting infection-derived peptides to immune system cells is crucial in initiating the immune response to infection. For instance, a study in Italy highlighted that HLA-C, which is considered a permissive allele for SARS-CoV-2, represented specific ligands for KIR2DL2 and KIR2DL3 [33].

These receptors inhibit NK cell activity, which plays a critical role in the initial immune defense against infection prior to the activation of T cell responses [34].

This study has certain limitations, including a small sample size due to stringent inclusion criteria, which required participants to be unvaccinated. Moreover, SSP HLA-typing is categorized as a low-resolution method. In epitope prediction, HLA affinity was examined only for the spike protein, which is one of the key proteins in COVID-19 virulence [35].

Most studies in this area have focused on examining HLA class II, reporting a positive correlation between the frequency of HLA-DRB1*15:01 and -DQB1*06:02 and severe COVID-19 infection. They have also reported another positive relationship between HLA-B*27:07 and the disease severity in COVID-19 patients [36]. However, an in-silico study reported a positive relationship between the HLA-A*02:01 allele and higher mortality rates. The occurrence of severe infections is affected by a range of variables including different genetic and environmental factors [37].

While these findings provide valuable insights, limitations such as the small sample size and focus on spike protein affinity emphasize the need for broader studies. Future research should incorporate larger cohorts, high-resolution HLA typing, and examination of other viral proteins to achieve a more comprehensive understanding of the genetic mechanisms underlying COVID-19 severity. Such investigations could pave the way for personalized therapeutic and preventive strategies based on HLA profiles, contributing to better management of current and future pandemics.

Conclusion

The present study highlights a significant association between HLA class I genotypes and COVID-19 severity within the Isfahan population. Specifically, the increased frequency of HLA-C*07 and HLA-A*01 among severe cases suggests a potential predisposing role for these alleles. Conversely, the protective roles of HLA-A*02 and HLA-A*03 align with findings from other populations. These results underscore the critical role of genetic factors in shaping individual susceptibility and clinical outcomes in infectious diseases, including COVID-19.

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