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Research article

*HLA-DRB1*04* may predict the severity of disease in a group of Iranian COVID-19 patients

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ABSTRACT

Human leukocyte antigen (HLA) genes with extreme diversity can make a contribution for individual variations to the immune response against SARS-COV-2 infection. This study aimed to explore the distributions of HLA class II alleles frequencies and their relations with disease severity in a group of Iranian COVID-19 patients. This prospective and case-control study was conducted on 144 COVID-19 patients including 46 cases with moderate form, 54 cases with severe and 44 cases with critical disease. *HLA-DRB1* and *-DQB1* allele families were determined by PCR-SSP method and compared between three groups of the patients and in comparison to 153 ethnic-matched healthy controls. The patients group showed lower frequencies of *HLA-DRB1*15* (OR = 0.57, P = 0.06), *DRB1*15 ~ DQB1*05* haplotype (P = 0.04) and *DRB1*15/DRB1*04* genotype (P = 0.04) in compare with healthy controls. Moderate COVID-19 patients had higher frequencies of *HLA-DRB1*04* (P = 0.03), *HLA-DRB1*10* (P = 0.05) and *DRB1*04/DRB1*11* genotype (P = 0.01). Also, a higher significantly frequency of *HLA-DRB1*03* allele group was observed in the critical patients versus controls (P = 0.01). Multiple logistic regression analysis revealed that the presence of *DRB1*04* allele group was negatively associated with development of severe and critical disease (OR: 0.289, P = 0.005). Our results indicate a possible contribution of some HLA class II alleles in disease severity and clinical features of COVID-19 disease.

1. Introduction

One year after the first report of severe acute respiratory syndrome coronavirus 2 (SARS-COV2) infection from Wuhan (China), over 89 million people have been infected globally and more than 1.9 million have died from this coronavirus disease 2019 (COVID-19) [1,2]. Global spreading of this new strain of beta coronavirus infection shows extremely variations in different geographical areas. More complicatedly, variations in the clinical picture and severity of COVID-19 infection fuels researchers with more unanswered questions regarding the pathogenesis of this viral infection [3]. Several factors including age, comorbidities (e.g. diabetes, obesity, cardiovascular diseases, etc.), host genetic backgrounds particularly immune-regulated genes such as human leukocyte antigen (HLA) genes, cytokine storm and prior exposure to other infectious agents are suggested to influences COVID-19 pathogenesis [2–4].

Among the multiple host genetic components involved in SARS-COV2 infection, immune-related genes have been suggested to play an important role in COVID-19 susceptibly or resilience, severity of disease and its outcomes. In this context, HLA genes with extreme diversity across different populations make a contribution for individual variations to the immune response against SARS-COV-2 infection [3].

Given the central role of HLA molecules in binding to viral antigenic peptides and presenting them to virus-specific cytotoxic T cells, HLA genes as the most polymorphic genetic system may function as susceptibility loci or even may confer protection against viral infections [3,4]. Amino acid variations inside the peptide-binding groove of HLA molecules results from genetic sequence variations in the exons coding this part of HLA molecules and these variations determine the positive or negative associations of HLA genes with any infectious diseases [4,5]. With this

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in mind, and based on the bioinformatical modeling identification of epitopes repertoire of SARS-COV2 for the induction of specific T cell-mediated antiviral responses, some HLA alleles are known to be the best-presenting (*A*02:02*, *B*15:03* and *C*12:03*) or poor-presenting (*A*25:01*. *B*46:01* and *C*01:02*) alleles for SARS-COV2 epitopes [4]. In addition, HLA association studies in COVID-19 patients in various populations revealed the positive associations of HLA-*DQB1*06*, *A*26*, *DRB1*15*, *DRB1*10* and negative associations of *A*02*, *B*44* and *C*05* alleles with COVID-19 [5], higher significantly frequencies of *B*15:27* and *C*07:29* [6], and a permissive role of *HLA-C*01* and *B*44* towards COVID-19 disease [7]. Alternatively, it has been reported that the higher frequencies of *HLA-A*02* allele group in certain population (North and Central Indians, Africans) might be related with lower incidence of SARS-COV2 infection in these areas [3].

Due to the inconsistent and conflicting results across different populations regarding the predisposing/protective role of HLA alleles for COVID-19 and their relations with severity and outcomes of this viral infection, we decided to explore the distributions of *HLA class II* alleles frequencies as well as their relations with the severity of disease in a group of Iranian COVID-19 patients.

2. Methods

2.1. Ethical statement

This prospective cohort and case-control study was conducted with the approval of our institutional Ethics Committee, Hamadan University of medical sciences (IR.UMSHA.REC.1399.1068) on 144 COVID-19 patients who admitted to Sina University Hospital.

2.2. Study subjects

2.2.1. Patients

Diagnosis and confirmation of SARS-COV2 infection were carried out based on the presence of viral RNA in nasopharyngeal swab samples (confirmed disease) and/or observation of the radiological changes in CT scan as well as known clinical presentations of COVID-19 for the suspected cases. The patients were classified into three groups according to the severity of disease. Group I) 46 cases were diagnosed with moderate form of the disease, group II) 54 cases with severe COVID-19 and group III) 44 cases with critical disease whom admitted to the intensive care unit (ICU). Classification of the patients was based on the local guidelines and radiological findings as follows: The radiologist evaluated all five lobes of the both lungs for the presence of inflammatory abnormalities including ground-glass opacities, mixed ground-glass opacities and consolidation, according to the method presented by Kunwei et al. [8,9]. In terms of the percentage of involvement, score of 0.0 to 4.0 was considered for each lobe: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%). Then, the total severity score (TSS) was calculated by summing the points of the five lobes which ranges from 0 to 20. A TSS equal to or<3 was considered as *mild* involvement, 4 to 7 as *moderate*, and equal to or more than 8 categorized in *severe* group [10]. Moreover, in the presence of imaging criteria of acute respiratory distress syndrome (ARDS) including ground-glass attenuation associated with traction bronchiolectasis or bronchiectasis, airspace consolidation associated with traction bronchiolectasis or bronchiectasis, crazy-paving pattern and honeycombing, or in the presence of complications such as pneumothorax, a TSS equal to or more than 8 was considered as *critical* disease [11].

2.2.2. Clinical and laboratory findings

The basic demographics and main clinical characteristics including age, gender, history of chronic diseases (e.g. diabetes, renal disease, liver disease, hypertension, cardiovascular disease), presence of malignancies and the history of other infectious diseases (e.g. HIV, HBV, etc) were documented using medical records for all patients. Also, laboratory data including complete blood count (CBC), neutrophil to lymphocyte ratio (NLR), serum levels of albumin, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), C-reactive protein (CRP), alkaline phosphatase (ALT), alanine transaminase (AST), d-dimer and ferritin, hemoglobin (Hb) concentration, erythrocyte sedimentation rate (ESR), platelet (PLT) count and pressure of arterial oxygen (Pa O2) were recorded at the time of admission to hospital. All patients received therapeutic interventions based on the current guidelines which consisted of antiviral drugs (Remdesivir or Favipiravir and Interferon beta-1a), anti-coagulant drugs (heparin or enoxaparin), anti-inflammatory agents (e.g. dexamethasone, methyl prednisolone and in some cases Actemra) and adjunct therapy including vitamin C, Zinc and nonstroidal anti-inflammatory drugs (NSAIDs). Moreover, antibiotics were used in the case of co-infection with bacteria particularly in the critical and severe patients.

2.3. HLA-DRB1 and -DQB1 genotyping

All patients were genotyped for *HLA-DRB1* and *HLA-DQB1* allele-families. Briefly, at the first step, genomic DNA was extracted from the peripheral blood samples using modified salting out methods [12]. Then, HLA-DNA typing was performed by PCR with sequence specific primers using low resolution HLA DR-DQ SSP kits (Olerup SSP®DQ-DR SSP Combi Tray, Stockholm, Sweden) as per manufacture's protocol. Following PCR amplification, the total product volume was loaded on to a 2.0% agarose gel stained with DNA safestain and viewed under UV transilluminator. Determining the *DRB1* and *DQB1* allele-families as defined by the first field for each allele (based on HLA nomenclature) were executed by SCORE software provided by the company (https://labproducts.caredx.com/software/score).

2.3.1. HLA DRB1 ~ DQB1 haplotype analysis

In addition, *HLA DRB1 ~ DQB1* haplotypes were assigned using an Expectation-Maximization (EM) Algorithm as implemented in the R statistical computing environment (https://cran.r-project.org/web/packages/gap/index.html).

2.4. Control subjects

We used the HLA data from an ethnically matched healthy control group (n = 150) which has been described in our previous HLA associated study in the same ethnic group [13] as well as more 3 control subjects to have a total number of 153 ethnically matched control for the case-control analyses.

2.5. Statistical analysis

HLA-DRB1 and *-DQB1* allele-families and haplotypes frequencies were derived by direct counting and then compared between the patients and controls using chi-square test with Yates' correction or Fisher's exact test as applicable. The Benjamini–Hochberg method was used to control false discovery rate (FDR) for multiple comparisons [14]. Assumptions of Hardy-Weinberg Equiliberium (HWE) in our HLA data for both patients and healthy controls groups was tested by GAP package in R-project (https://cran.r-project.org/web/packages/gap/index.html). The risk contributed by the haplotype and genotype were assessed by calculation of odds ratio (OR) with 95% confidence interval. Also, paired-*t*-test was

performed to analyze quantitative data. All data was analyzed using the SPSS V.16.0. A probability value and FDR corrected P values less than 0.05 were considered as statistically significant.

3. Results

Comparison of the baseline characteristics as well as some clinical and para-clinical data between three subgroups of the patients are summarized in Table 1. We observed the significant differences between moderate, severe and critical COVID-19 patients in terms of the mean age (P < 0.001), pressure of arterial O2 saturation (P < 0.001), pulse rate (PR) (P = 0.01), percentages of neutrophils and lymphocytes (P < 0.001), neutrophil to lymphocyte ratio (NLR) (P < 0.0001), Hb concentration (P = 0.001), ESR (P = 0.002), CRP and albumin levels (P < 0.001), AST (P = 0.04), ALP (P = 0.007), LDH (P < 0.001), BUN (P = 0.001) and number of positive cases for real-time PCR test (P = 0.03) (Table 1). Moreover, we observed significant differences for mortality rates, presence of diabetes, hypertension, malignancies, and clinical manifestations (myalgia, dyspnea, olfactory dysfunction and cough) between three subgroups of the patients (Table 1). There were no significant differences for the mean of RR, SBP, DBP, BMI, WBC count, PLT, ALT, Cr, CPK, PCO2, Bilirubin, PT, INR, and PTT between three subgroups of the patients.

We examined the *HLA class II* allele-families prevalence among 144 COVID-19 patients in terms of the severity of disease and in comparison to healthy controls. Testing for deviation from HWE for DRB1 loci did not show significant departures in the healthy controls (P = 0.406) and patients (P = 0.259) groups. The patients group showed lower frequencies of *HLA-DRB1*15* (OR = 0.57, P = 0.06, Table 2), corresponding haplotype *DRB1*15 ~ DQB1*05* (P = 0.04, Suppl Table 1) and *DRB1*15/DRB1*04* genotype (P = 0.04, Suppl Table 2) in compare with healthy controls. Comparison of the alleles frequencies between three subgroups of the patients revealed that moderate COVID-19 patients had higher frequencies of *HLA-DRB1*04* (P = 0.03) and *HLA-DRB1*10* allele-groups (P = 0.05, Table 3), *DRB1*04/DRB1*11* genotype (P = 0.01, Suppl Table 3), as well as *DRB1*04 ~ DQB1*03* and *DRB1*10 ~ DQB1*05* haplotypes (P = 0.03 and P = 0.05 respectively, Table 4) versus severe and critical patients. However, after correction of the probability values by multiple comparison, we did not find significant differences for the frequencies of alleles and haplotypes between three subgroups of the patients (Tables 3 and 4). Moreover, we found a higher significantly frequency of *HLA-DRB1*03* allele-group in the critical subgroup of the patients than healthy controls (P = 0.01, Suppl Table 4). No significant differences were found for the frequencies of *HLA-DQB1* allele-families either between the patients and controls or within the COVID-19 patients' subgroups (Suppl Tables 5 and 6).

Table 1
Demographics and the significantly clinical characteristics among three subgroups of the COVID-19 patients based on disease severity.

Variables	Moderate N = 46	Severe N = 54	Critical N = 44	P-Value
Age (year)	55.26 ± 19.06 (16–93)	62.09 ± 15.97 (27–86)	70.61 ± 12.65 (38–95)	<0.001
Gender (Female/Male)	23/23	22/32	20/24	0.65
Pulse rate	91.34 ± 10.94 (74–118)	86.03 ± 13.67 (60–130)	93.57 ± 12.53 (71–125)	0.01
First O2 saturation (mmhg)	83.57 ± 11.78 (58–92)	82.60 ± 11.73 (62–90)	73.00 ± 11.71 (54–90)	<0.001
Last O2 saturation (mmhg)	92.29 ± 9.17 (34–98)	92.91 ± 3.16 (78–98)	76.93 ± 20.04 (31–96)	<0.001
Neutrophil (%)	67.79 ± 12.55 (21–93)	69.85 ± 10.18 (50–90)	79.30 ± 11.76 (41–95)	<0.001
Lymphocyte (%)	27.02 ± 12.32 (2–70)	25.57 ± 10.01 (5–44)	17.07 ± 10.56 (3–54)	<0.001
NLR	3.14 ± 2.09 (0.30–11.25)	3.74 ± 3.17 (1.23–17.60)	7.42 ± 6.27 (0.75–31)	<0.0001
Hemoglobin (g/L)	14.16 ± 1.36 (10.8–17.3)	14.49 ± 1.54 (11.7–18.7)	13.15 ± 2.36 (5.8–18.1)	0.001
ESR (mm/h)	51.57 ± 48.65 (4–120)	40.00 ± 16.41 (24–64)	55.36 ± 35.01 (6–105)	0.02
CRP (mg/L)	7.42 ± 4.90 (2–16)	32.32 ± 23.79 (0.90–71.00)	105 ± 138.62 (21–265)	<0.001
Albumin (g/L)	3.99 ± 0.41 (2.80–4.70)	3.87 ± 0.44 (1.80–4.50)	3.38 ± 0.57 (2.30–4.60)	<0.001
AST (U/L)	31.55 ± 15.83 (14–87)	45.51 ± 66.39 (12–460)	63.60 ± 70.11 (11–416)	0.04
ALP (U/L)	183.64 ± 80.47 (50–535)	185.61 ± 103.38 (60–629)	249.04 ± 126.24 (101–857)	0.007
LDH (U/L)	458.65 ± 121.13 (136–727)	529.41 ± 199.20 (168–1349)	937.65 ± 444.25 (278–2283)	<0.001
BUN (mmol/L)	17.58 ± 14.27 (5–79)	16.52 ± 6.76 (7–34)	25.51 ± 14.60 (8–85)	0.001
PCR (Pos/Neg)	19/27	21/33	28/16	0.03
Myalgia (Yes/No)	11/34	18/36	34/10	<0.0001
Dyspnea (Yes/No)	27/19	36/18	42/2	0.0001
Cough (Yes/No)	23/23	32/22	38/6	0.0008
Olfactory dysfunction (Yes/No)	4/42	1/53	20/22	<0.0001
Diabetes (Yes/No)	12/34	8/46	21/23	0.001
Hypertension (Yes/No)	21/25	17/37	25/19	0.04
Malignancy (Yes/No)	0	0	3/0	0.03
30-day Mortality rate	4/42	8/46	19/25	0.0001

Note: All quantitative data are presented as mean ± SD and range. NLR: Neutrophil-to-lymphocyte ratio, ESR: Erythrocyte sedimentation rate, AST: Aspartate transaminase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, PCR: Polymerase chain reaction test.

Table 2
Comparison of the *HLA-DRB1* allele-families frequencies between Covid-19 patients (n = 144) and healthy controls (n = 153).

DRB1 allele-groups	Covid-19 patients 2*n* = 288 (%)	Control 2*n* = 306 (%)	OR [95% CI]	*P*-value
*DRB1*01*	16 (5.5)	20 (6.5)	0.841 [0.427 to 1.657]	0.74
*DRB1*03*	40 (13.8)	29 (9.5)	1.540 [0.927 to 2.559]	0.12
*DRB1*04*	31 (10.7)	33 (10.8)	0.997 [0.593 to 1.676]	1.00
*DRB1*07*	30 (10.4)	30 (9.8)	1.069 [0.627 to 1.824]	0.91
*DRB1*08*	3 (1.0)	9 (2.9)	0.347 [0.093 to 1.296]	0.17
*DRB1*09*	2 (0.7)	4 (1.3)	0.528 [0.096 to 2.906]	0.73
*DRB1*10*	5 (1.7)	9 (2.9)	0.583 [0.193 to 1.760]	0.48
*DRB1*11*	67 (23.2)	66 (21.5)	1.102 [0.749 to 1.621]	0.69
*DRB1*12*	1 (0.3)	3 (0.9)	0.351 [0.036 to 3.402]	0.65
*DRB1*13*	45 (15.6)	39 (12.7)	1.267 [0.798 to 2.013]	0.37
*DRB1*14*	10 (3.4)	17 (5.5)	0.611 [0.275 to 1.358]	0.30
*DRB1*15*	**23 (7.9)**	**40 (13.1)**	**0.577 [0.336 to 0.990]**	**0.06**
*DRB1*16*	15 (5.2)	7 (2.3)	2.346 [0.942 to 5.842]	0.09

Note: Comparisons for the alleles with low frequency (2n < 5) were calculated based on Two-tailed P values by Fisher's exact test.

Table 3
Distributions of *HLA-DRB1* allele-families among three subgroups of the patients according to the disease severity.

DRB1 allele-groups	Moderate 2n = 92	Severe 2n = 108	Critical 2n = 88	*P-value*	P_c [§]
*DRB1*01*	3 (3.2)	7 (6.4)	6 (6.8)	0.55	0.62
*DRB1*03*	13 (14.1)	10 (9.2)	17 (19.3)	0.20	0.57
*DRB1*04*	16 (17.3)	10 (9.2)	5 (5.6)	**0.01**	0.13
*DRB1*07*	7 (7.6)	14 (12.9)	9 (10.2)	0.37	0.60
*DRB1*08*	0 (0.0)	3 (2.7)	0 (0.0)	0.18	0.57
*DRB1*09*	1 (1.0)	0 (0.0)	1 (1.1)	0.54	0.62
*DRB1*10*	4 (4.3)	1 (0.9)	0 (0.0)	**0.05**	0.32
*DRB1*11*	22 (23.9)	26 (24.0)	19 (21.5)	0.78	0.78
*DRB1*12*	0 (0.0)	0 (0.0)	1 (1.1)	0.31	0.57
*DRB1*13*	14 (15.2)	14 (12.9)	17 (19.3)	0.30	0.57
*DRB1*14*	2 (2.1)	6 (5.5)	2 (2.2)	0.31	0.57
*DRB1*15*	5 (5.4)	10 (9.2)	8 (9.0)	0.58	0.62
*DRB1*16*	5 (5.4)	7 (6.4)	3 (3.4)	0.57	0.62

§ **:** False discovery rate (FDR)-corrected P values for multiple testing using the Benjamini-Hochberg method. Comparisons for the alleles with low frequency (2n < 5) were calculated based on Two-tailed P values by Fisher's exact test.

Table 4
Distributions of *HLA-DRB1 ~ DQB1* haplotypes among three subgroups of the patients according to the disease severity.

HLA haplotypes	Moderate 2n = 92	Severe 2n = 108	Critical 2n = 88	P-value	P_C [§]
*DRB1*01 ~ DQB1*05*	3 (3.2)	7 (6.4)	6 (6.8)	0.58	0.66
*DRB1*03 ~ DQB1*02*	13 (14.1)	10 (9.2)	17 (19.3)	0.20	0.60
*DRB1*04 ~ DQB1*03*	**16 (17.3)**	**10 (9.2)**	**5 (5.6)**	**0.03**	0.15
*DRB1*07 ~ DQB1*02*	6 (6.5)	13 (12.0)	6 (6.8)	0.17	0.60
*DRB1*07 ~ DQB1*03*	1 (1.0)	1 (0.9)	3 (3.4)	0.34	0.63
*DRB1*08 ~ DQB1*04*	0 (0.0)	3 (2.7)	0 (0.0)	0.18	0.60
*DRB1*09 ~ DQB1*03*	1 (1.0)	0 (0.0)	1 (1.1)	0.54	0.66
*DRB1*10 ~ DQB1*05*	**4 (4.3)**	**1 (0.9)**	**0 (0.0)**	**0.05**	0.37
*DRB1*11 ~ DQB1*03*	22 (23.9)	26 (24.0)	19 (21.5)	0.78	0.78
*DRB1*12 ~ DQB1*03*	0 (0.0)	0 (0.0)	1 (1.1)	0.31	0.63
*DRB1*13 ~ DQB1*03*	2 (2.1)	4 (3.7)	4 (4.5)	0.62	0.66
*DRB1*13 ~ DQB1*06*	12 (13.0)	10 (9.2)	13 (14.7)	0.38	0.63
*DRB1*14 ~ DQB1*05*	2 (2.1)	6 (5.5)	2 (2.2)	0.31	0.63
*DRB1*15 ~ DQB1*06*	5 (5.4)	10 (9.2)	8 (9.0)	0.58	0.66
*DRB1*16 ~ DQB1*05*	5 (5.4)	7 (6.4)	3 (3.4)	0.57	0.66

§ **:** False discovery rate (FDR)-corrected P values for multiple testing using the Benjamini-Hochberg method. Comparisons for the haplotypes with low frequency (2n < 5) were calculated based on Two-tailed P values by Fisher's exact test.

Analysis of the distributions of *HLA-DRB1* and *-DQB1* allele-families in all patients based on NLR showed that all patients carrying *DRB1*10* allele-group had NLR < 3.0 (P = 0.02, Table 5) and patients with lymphopenia showed higher frequency of *DRB1*15* allele-group compared to those cases without lymphopenia (P = 0.05, Table 6). Also, multiple logistic regression analysis revealed that the presence of *DRB1*04* allele-group was negatively associated with development of severe and critical disease (OR: 0.289, P = 0.005) and having lymphopenia was directly correlated with developing severe and critical COVID-19 disease (OR: 3.612, P = 0.03, Table 7).

4. Discussion

Given the central role of HLA molecules for the stimulation and induction of T cell-mediated anti-viral immunity, it is speculative

Table 5
Comparison of the *HLA-DRB1* allele-families frequencies among the patients according to the mean neutrophil-to-lymphocyte ratio (NLR).

*DRB1** Allele-families (n)	NLR		p-Value	OR (95% CI)
	≤3 (n = 70)	>3 (n = 73)		
*DRB1*01 (15)*	8 (53.3%)	7 (46.7%)	0.78	0.82 (0.28–2.40)
*DRB1*03 (36)*	17 (47.2%)	19 (52.8%)	0.84	1.09 (0.51–2.33)
*DRB1*04 (30)*	14 (46.6%)	16 (53.4%)	0.83	1.12 (0.50–2.51)
*DRB1*07 (28)*	15 (53.5%)	13 (46.5%)	0.67	0.79 (0.34–1.81)
*DRB1*08 (2)*	1 (50.0%)	1 (50.0%)	1.00	0.95 (0.05–15.62)
*DRB1*09 (2)*	0 (0.0%)	2 (100.0%)	0.49	1.02 (0.98–1.06)
*DRB1*10 (5)*	5 (100.0%)	0 (0.0%)	**0.02**	0.92 (0.87–0.99)
*DRB1*11 (65)*	32 (49.2%)	33 (50.8%)	1.00	0.98 (0.50–1.89)
*DRB1*12 (1)*	1 (100.0%)	0 (0.0%)	0.49	0.98 (0.95–1.01)
*DRB1*13 (41)*	18 (43.9%)	23 (56.1%)	0.46	1.32 (0.64–2.75)
*DRB1*14 (10)*	5 (50.0%)	5 (50.0%)	1.00	0.95 (0.26–3.45)
*DRB1*15 (21)*	8 (38.0%)	13 (62.0%)	0.34	1.67 (0.65–4.34)
*DRB1*16 (14)*	5 (35.7%)	9 (64.3%)	0.40	1.82 (0.58–5.75)

Note: Comparisons for the alleles with low frequency (2n < 5) were calculated based on Two-tailed P values by Fisher's exact test.

Table 6
Comparison of the *HLA-DRB1* allele-families frequencies based on developing lymphopenia in the patients group.

DRB1 allele-families (n)	Lymphopenia		P-Value	OR (95% CI)
	Positive (%)	Negative (%)		
*DRB1*01 (15)*	3 (20.0%)	12 (80.0%)	0.17	0.39 (0.10–1.45)
*DRB1*03 (36)*	15 (41.6%)	21 (58.4%)	0.55	1.29 (0.59–2.80)
*DRB1*04 (30)*	11 (36.6%)	19 (63.4%)	1.00	0.97 (0.42–2.25)
*DRB1*07 (28)*	9 (32.1%)	19 (67.9%)	0.66	0.76 (0.31–1.83)
*DRB1*08 (2)*	1 (50.0%)	1 (50.0%)	1.00	1.71 (0.10–27.94)
*DRB1*09 (2)*	1 (50.0%)	1 (50.0%)	1.00	1.71 (0.10–27.94)
*DRB1*10 (5)*	0 (0.0%)	5 (100.0%)	0.15	0.94 (0.89–0.99)
*DRB1*11 (65)*	24 (36.9%)	41 (63.1%)	1.00	0.98 (0.5–1.95)
*DRB1*12 (1)*	0 (0.0%)	1 (100.0%)	1.00	0.98 (0.96–1.01)
*DRB1*13 (41)*	16 (39.0%)	25 (61.0%)	0.84	1.12 (0.53–2.37)
*DRB1*14 (10)*	3 (30.0%)	7 (70.0%)	0.74	0.71 (0.17–2.87)
*DRB1*15 (21)*	12 (57.1%)	9 (42.9%)	**0.05**	2.63 (1.02–6.75)
*DRB1*16 (14)*	7 (50.0%)	7 (50.0%)	0.38	1.80 (0.59–5.46)

Note: Comparisons for the alleles with low frequency (2n < 5) were calculated based on Two-tailed P values by Fisher's exact test.

Table 7
Multiple logistic regression analysis to evaluate the prognostic values of potential variables for development of severe and critical forms of COVID-19 disease.

Variables	Odds Ratio (OR)	95% C.I.	P Value
*HLA-DRB1*04*	0.289	0.21–0.69	0.005
*HLA-DRB1*10*	0.125	0.01–1.22	0.07
Lymphopenia	3.612	1.13–11.49	0.03
NLR*	0.696	0.24–1.99	0.50

* NLR: Neutrophil-to-lymphocyte ratio.

that different HLA alleles may determine the genetic susceptibility to or possibly protection against COVID-19 and they can be related with severity, prognosis and outcome of diseases [2,4,15]. In the present study, we sought to find out any association of *HLA-DRB1 and -DQB1* alleles with disease's susceptibility and severity in a group of Iranian COVID-19 patients.

We found the higher significantly frequencies of *HLA-DRB1*04* and *DRB1*10* allele-families as well as their corresponding haplotypes, *DRB1*04 ~ DQB1*03* and *DRB1*10 ~ DQB1*05*, among moderate cases compared to severe and critical patients (Tables 3 and 4). These associations were tested by multiple logistic regression analysis and *DRB1*04* was shown to be inversely correlated with developing of the severe and critical forms of COVID-19. Moreover, similar analysis revealed a direct correlation between lymphopenia and progression of severe/critical forms of the disease (Table 7). Also, a lower significant NLR was observed in the patients carrying *DRB1*10* allele-group. These results could be implicative for the possibly contributions of specific *HLA class II* alleles in the induction of more appropriate anti-viral responses in this group of the patients. In this context, and in support of our findings, *in silico* analysis of MHC binding capacity for SARS-COV2 epitopes have shown that some immunogenic epitopes of the virus can be presented by distinct HLA class I (*A*02:01*, *B*40:01*) and class II (*DRA*01:01*, *DRB1*07:01* and *DRB1*04:01*) alleles [16]. Although, our findings need to be confirmed by further investigations using larger cohorts of patients to find out the exact relationship between DRB1*04 and severity of COVID-19 disease. Our study was underpowered to find any significant association between HLA class II alleles' frequencies and COVID-19 susceptibility, and the only marginally significant difference was found for DRB1*15 allele (P = 0.06). Whereas, study of 82 Chinese COVID-19 patients revealed the higher frequencies of *HLA-C*07:29*, *C*08:01G*, *B*15:27*, *B*40:06*, *DRB1*04:06* and *DPB1*36:01* alleles and lower frequencies of *DRB1*12:02* and *DPB1*04:01* alleles in the patients group than healthy controls. However, the only significant difference after correcting the P values were shown for *B*15:27* and *C*07:29* alleles in above study [6].

Our results depicted a trend toward higher frequency of *DRB1*15* among patients with lymphopenia (P = 0.05) which also could be implicative for a possibly detrimental role of this allele in COVID-19 immunopathogenesis. Similarly, Poulton *et al.* study showed an impaired presentation of viral epitopes in those COVID-19 patients carrying *HLA-DRB1*15:01*, *DQB1*06:02*, *DRB1*10* and *HLA-A*26* alleles [5]. This in turn, may lead to an inefficient T cell response and consequently insufficient antibody response, which is necessary for neutralization and elimination of the virus in synergy with T cell medicated immunity [5,17]. Likewise, Kachuri et al. study in UK population revealed higher

incidence of SARS-COV2 positivity among individuals carrying *DRB1*15:01* and *DQB1*06:02* alleles [17].

In contrast to these findings, we found higher frequencies of DRB1*10 among moderate patients versus severe to critical patients. Elucidation of the consistent results for *DRB1*15* allele-group and contradictory results for *DRB1*10* allele-group may be indicative for more complexities for the role of HLA molecules in immunopathogenesis of this viral infection. Hence, our results must be interpreted with caution and these preliminary findings deserves to be explored in the larger cohorts of COVID-19 patients and more importantly in terms of the mechanistic view in our population as well as in other ethnic groups. It is speculative that the HLA molecules make a contribution either in disease protection or in the severity of clinical disease probably via facilitation of the virus entry into the cells through HLA molecules as an attachment factor for the virus protein [18], and through up regulation of HLA-DR expression in the lung tissue of COVID-19 patients [19].

Observation of the potential link between *HLA-DRB1*04* and *DRB1*10* allele-families and severity of COVID-19 disease in our study even by recruiting small number of patients, prompted us to further explore this association by analyzing other HLA loci and probably other genetic loci in COVID-19 patients. This, in turn, might be useful for determining the potential prognostic value of HLA genes, better understanding of the immunopathogenesis of this new beta coronavirus and better management of COVID-19 patients.

Considering the patterns of the main clinical characteristics among three subgroups of the COVID-19 patients, we found higher significantly incidences of comorbidities such as diabetes, hypertension, and cancer among critical cases than moderate to severe patients. Moreover, critical patients had higher incidence of myalgia, dyspnea, cough and olfactory dysfunction in compare with moderate and severe cases (Table 1). With regard to the laboratory data, critical patients also showed significant differences for BUN, LDH, ALP, AST, CRP, ESR, NLR, neutrophil and lymphocyte counts and number of positive RT-PCR test compared to moderate and severe patients groups. Our findings in this regard are in line with previous reports that demonstrated the significant correlation between the presence of comorbidities and elevated NLR and CRP and poor clinical outcomes in COVID19 patients [20,21].

In conclusion, the results of this preliminary study on a small group of Iranian COVID-19 patients indicates a possible link between some HLA class II allele-families, *DRB1*04*, and disease severity. However, our findings must be interpreted with caution, because of some important limitations in the present study such as low number of analyzed samples and lack of high-resolution typing for the HLA alleles. Therefore, the real nature of this link and particularly in terms of the mechanistic view, needs to be clarified by further experimental studies in ours and other populations. Finally, these observational study may have a potential clinical implication for defining genetic predictors of disease severity and possibly genetic determinant to identify at-risk individuals. This, in turn, may provide the important insights for future vaccination strategies and better clinical management of this viral infection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2021.07.004.

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