Association Between Human Leukocyte Antigen and COVID-19 Severity

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Abstract- In the last days of 2019, a new coronavirus emerged in Wuhan, China, and less than three months its disease, now called COVID-19, was announced a global pandemic by WHO. COVID-19 usually causes respiratory symptoms and can lead to more severe conditions like ARDS. HLA has a crucial role in regulating the immune system; thus, different HLA allele types can be a protective or risk factor for some diseases, so we aimed to find such associations to determine whether some alleles can predict susceptibility or resistibility to COVID-19 and finally facilitate vaccine development. In this case-control study, 15 admitted COVID-19 cases with severe symptoms and ten individuals with mild COVID-19 symptoms were enrolled in the case and control groups, respectively. They were genotyped for HLA A/B/DR loci using a low-resolution HLA typing test. These alleles were more prevalent in case (severe COVID-19) group: A*24 (53.33% vs 10%), B*50 (20% vs 10%), B*55 (20% vs 10%), DRB1*04 (40% vs 20%) and DRB1*11 (53.33% vs 30%) but the difference was only statically significant in A*24 allele (*P*=0.027; odd ratio=10.286). A*24 was also more prevalent in all patients than the general population in Iran. A*24 was the only allele more prevalent in severe COVID-19 cases with statistical significance. This allele was reported to be a risk factor for such autoimmune diseases as type 1 diabetes, myasthenia gravis, and systemic lupus erythematosus, which may be related to reported immune system hyperresponsiveness in severe COVID-19 cases.

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Introduction

Coronaviruses are enveloped RNA viruses that are categorized in the coronaviridae family. These viruses can infect various species, and their presentations in humans are usually mild to moderate respiratory symptoms, but SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (middle east

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respiratory syndrome coronavirus) outbreaks in china 2002 and the middle east 2012 respectively showed that this virus could be a serious threat to human health (1).

On December 31th 2019, several pneumonia cases with unknown origin in Wuhan reported to the world health organization (WHO) that their cause was confirmed to be a coronavirus on January 7th, 2020, and now it is named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (2). From then, COVID-19 (Coronavirus disease 2019) rapidly spread across the globe, and it was officially announced by WHO (world health organization) as a pandemic on March 11th, 2020 (3). As of August 23rd, 2020, near 23 million were diagnosed with COVID-19, and it took near 800,000 victims (4). It is usually presented with fever (87.3%; 95% CI=0.838-0.909), cough (58.1%; 0.502-0.660), dyspnea (38.3%; 0.246-0.520), myalgia or fatigue (35.5%; 0.253-0.456) and chest discomfort (31.2%; 0.024-0.648). The main imaging finding was bilateral pneumonia (75.7%, 0.639-0.871) and ground-glass opacification (69.9%, 0.602-0.796). Among the patients, the incidence of required intensive care unit (ICU) was (29.3%, 0.190-0.395), the incidence of acute respiratory distress syndrome (ARDS) was (28.8%, 0.147-0.429), the multiple organ dysfunction syndromes (MODS) was (8.5%, -0.008-0.179), and the case fatality rate of patients with COVID-19 was (6.8%, 0.044-0.093) (3) COVID-19 diagnosis is usually confirmed by RT-PCR (real-time fluorescence polymerase chain reaction) (5), Chest CT scan can be an acceptable method to diagnose early cases even without clinical symptoms in addition to cases with negative PCR results (2).

HLA (human leukocyte antigen) plays an important role in immune system regulation. These molecules constantly interact with T lymphocyte receptors to distinguish non-self from self-antigens. HLA genes are in MHC (major histocompatibility complex) region,- which is located on chromosome 6p21.3; there are more than 20000 alleles in MHC encoding Class I HLA-A, HLA-B, and HLA-C loci, and Class II HLA-DR, HLA-DQ, and HLA-DP loci. Determining HLA type has a crucial role in solid organ transplantation, hematopoietic stem cell transplantation, transfusion medicine for refractory platelet patients, and the diagnostic workup of various disease associations and pharmacogenomics applications. Molecule methods of HLA testing can be done on different levels or as is called "resolutions." Low-resolution testing is usually used for solid organ transplant and disease associations, but hematopoietic stem cell transplantation needs high-resolution testing to determine gene sequence in detail (6).

After MERS-CoV and SARS-CoV outbreaks, there were researches to find an association between HLA gene alleles and the severity of these diseases in individuals (7,8). Due to the significance of COVID-19 as a global threat on the one hand and inadequate studies on this subject considering disease novelty, on the other hand, we aimed to find such imperative associations to predict patient's prognosis and finally help developing an effective vaccine for this widespread disease.

Materials and Methods

Study design and setting

The present study was derived from the Sina Hospital COVID-19 Registry (SHCo-19R), which has been described previously in detail regarding study design, diagnostic, and management protocols (9). In brief, SCHo-19R is an ongoing, prospective, hospital-based registry of patients with COVID-19 presenting to the emergency department of Sina Hospital, which is a primary COVID-19 referral center affiliated to Tehran University of Medical Sciences (TUMS) in south Tehran, Iran. This is a case/control study conducted at Sina hospital in Tehran, Iran. This project was approved by the COVID-19 research committee of Sina Hospital and the ethical committee of TUMS and was conducted under the Declaration of Helsinki (10). All patients have signed written informed consent forms upon admission before enrollment.

According to WHO interim guidance, consecutive hospitalized patients over 18 years of age with a diagnosis of COVID-19 confirmed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) of the oropharyngeal swab or endotracheal samples were enrolled in the present study (11). According to the Iranian national committee of COVID-19, we also enrolled highly suspicious patients, including individuals with a compatible chest computed tomography (CT) scan finding including ground-glass opacity alone or ground-glass opacity accompanied by consolidation, not fully explained by volume overload, lobar or lung collapse, or nodules along with the history compatible with COVID-19 (12).

Case (severe) group were chosen from COVID-19 confirmed patients under 70 years without severe comorbidity admitted to ICU (intensive care unit) from March 15^{th,} 2020 to June 15^{th,} 2020, which met at least one of the following criteria: dyspnea, respiratory rate ≥30/min, oxygen saturation ≤93%, >50% lung involvement on imaging, respiratory failure, shock, or multiorgan damage. This criterion was modified using

Wu and colleagues' definition (13).

Outpatients with confirmed COVID-19 without severe criteria above attending our hospital clinics were enrolled in the control (mild) group.

Data collection

We obtained admitted patient's epidemiological, clinical, laboratory, radiological, and medical management data using Sina hospitals' electronic COVID-19 registry. Registered data included demographics, symptoms onset, clinical presentation (i.e., fever, dyspnea, fatigue, cough, anosmia, pharyngodynia, myalgia, diarrhea, nausea/vomiting, abdominal pain, and headache), exposure history, past medical history, drug history, laboratory results, chest CT scans, clinical management, hospital stay, clinical course, and outcome. Outpatient data were taken based on the medical history, including demographics, symptoms onset, clinical presentation, exposure history, past medical history, and drug history, then they were followed up by telephone after two weeks.

HLA allele typing

We used 2cc EDTA (ethylenediaminetetraacetic acid), added whole blood from each patient, extracted DNA (deoxyribonucleic acid) using FavorPrep™ Blood Genomic DNA Extraction Kit by Favorgen® with catalog number FABG001, and DNA quality and quantity were determined using NanoDrop™ device by DeNovix®. Then we used the Olerup SSP® low-resolution A/B/DR kit to multiply A, B, and DR alleles in Bio-Rad T100™ thermal cycler. The final PCR product was fixed on gel along Ladder100, and its picture was taken by Bio-Rad Gel Doc. At last, we analyzed the picture using Helmberg-SCORE™ software version 5.00.80.02T to find A/B/DR alleles.

Statistical analysis

Each allele frequency was calculated and compared between case and control groups using Pearson chi-square and Fisher exact test with Statistical significance under 0.05. All analyzed by IBM SPSS® version 26.

Results

Twenty patients enrolled in our study with an average age of 52.12 (18-70), including 19 men and six women. Fifteen patients were in the case (severe) group with an average age of 51.80 (min=18, max=70), including 11 men and four women; 10 patients were in the control (mild) group with an average age of 52.60 (min=44, max=65), including eight men and two women (Table 1). The most prevalent alleles in all patients were DRB1*11, A*02, B*35, A*24, and DRB1*04; in the control group: A*02, B*35, A*11, A*32, and DRB1*7 and in case of group: A*24, DRB1*11, B*35, DRB1*04 and A*02 (Table 2). In comparison these alleles were more prevalent in case group: A*24 (53.33% vs 10%), B*50 (20% vs 10%), B*55 (20% vs 10%), DRB1*04 (40% vs 20%) and DRB1*11 (53.33% vs 30%) but the difference was only statically significant in A*24 allele (*P*=0.027; odd ratio=10.286) (Table 3).

Table 1. Patients' characteristics

Characteristic		Control Group (N=10)	Case Group (N=15)
Demographics	**Age**	Mean=52.6 (Range=44-65)	Mean=51.8 (Range=18-70)
	Sex	8 men, 2 women	11 men, four women
Comorbidities	**Diabetes Mellitus**	0 (0%)	2 (13.3%)
	Hypertension	20 (20%)	4 (26.6%)
	Ischemic heart disease	0 (0%)	2 (13.3%)
	Asthma	0 (0%)	1 (6.6%)
	Malignancy	0 (0%)	1 (6.6%)
Clinical outcomes	**Hospital length of stay (days)**	-	Mean=22 (Range=5-70)
	Mortality	0 (0%)	5 (33.3%)

Table 2. Allele's frequency

Case group		Control group		All patients	
A*02	60.00%	A*24	53.33%	DRB1*11	44.00%
B*35	40.00%	DRB1*11	53.33%	A*02	40.00%
A*11	30.00%	B*35	40.00%	B*35	40.00%
A*32	30.00%	DRB1*04	40.00%	A*24	36.00%
DRB1*07	30.00%	A*02	26.67%	DRB1*04	32.00%
DRB1*11	30.00%	A*11	26.67%	A*11	28.00%
DRB1*13	30.00%	A*30	20.00%	A*32	24.00%
DRB1*14	30.00%	A*32	20.00%	DRB1*13	24.00%
B*18	20.00%	B*50	20.00%	DRB1*14	20.00%
B*38	20.00%	B*55	20.00%	B*50	16.00%
B*44	20.00%	DRB1*01	20.00%	B*55	16.00%
DRB1*03	20.00%	DRB1*13	20.00%	DRB1*03	16.00%
DRB1*04	20.00%	A*01	13.33%	DRB1*07	16.00%
A*01	10.00%	B*14	13.33%	A*01	12.00%
A*24	10.00%	B*15	13.33%	A*30	12.00%
A*26	10.00%	B*40	13.33%	B*15	12.00%
A*29	10.00%	B*51	13.33%	B*18	12.00%
A*66	10.00%	DRB1*03	13.33%	B*38	12.00%
B*07	10.00%	DRB1*14	13.33%	B*44	12.00%
B*15	10.00%	DRB1*15	13.33%	DRB1*01	12.00%
B*27	10.00%	A*03	6.67%	DRB1*15	12.00%
B*47	10.00%	A*23	6.67%	B*07	8.00%
B*49	10.00%	A*31	6.67%	B*14	8.00%
B*50	10.00%	A*33	6.67%	B*40	8.00%
B*52	10.00%	B*07	6.67%	B*49	8.00%
B*55	10.00%	B*13	6.67%	B*51	8.00%
B*58	10.00%	B*18	6.67%	B*58	8.00%
DRB1*08	10.00%	B*38	6.67%	DRB1*08	8.00%
DRB1*10	10.00%	B*44	6.67%	A*03	4.00%
DRB1*15	10.00%	B*49	6.67%	A*23	4.00%
A*03	0.00%	B*53	6.67%	A*26	4.00%
A*23	0.00%	B*57	6.67%	A*29	4.00%
A*30	0.00%	B*58	6.67%	A*31	4.00%
A*31	0.00%	DRB1*07	6.67%	A*33	4.00%
A*33	0.00%	DRB1*08	6.67%	A*66	4.00%
B*13	0.00%	DRB1*12	6.67%	B*13	4.00%
B*14	0.00%	A*26	0.00%	B*27	4.00%
B*40	0.00%	A*29	0.00%	B*47	4.00%
B*51	0.00%	A*66	0.00%	B*52	4.00%
B*53	0.00%	B*27	0.00%	B*53	4.00%
B*57	0.00%	B*47	0.00%	B*57	4.00%
DRB1*01	0.00%	B*52	0.00%	DRB1*10	4.00%

Table 3. Allele frequency comparison between groups

Allele	Case group	Control group	*P*†	Odd ratio
A*02	40.00%	40.00%	0.122	0.242 (0.44-1.335)
A*11	26.67%	30.00%	1	0.848 (0.144-4.990)
A*24	53.33%	10.00%	0.027	10.286 (1.030-102.753)
A*32	20.00%	30.00%	0.653	0.583 (0.092-3.717)
B*35	40.00%	40.00%	1	1.000 (0.195-5.121)
B*50	20.00%	10.00%	0.626	2.250 (0.200-25.369)
B*55	20.00%	10.00%	0.626	2.250 (0.200-25.369)
DRB1*03	13.33%	20.00%	1	0.615 (0.072-5.276)
DRB1*04	40.00%	20.00%	0.402	2.667 (0.414-17.169)
DRB1*07	6.67%	30.00%	0.267	0.167 (0.015-1.909)
DRB1*11	53.33%	30.00%	0.414	2.667 (0.492-14.461)
DRB1*13	20.00%	30.00%	0.653	0.583 (0.092-3.717)
DRB1*14	13.33%	30.00%	0.358	0.359 (0.048-2.683)

†Statistically significant *P* are bolded

Discussion

In our study, A*24 was the only allele more prevalent in severe COVID-19 cases with statistical significance. This allele is very common in southeast Asia, especially in Taiwanese people and Papua New Guinea (near 80%) (14). In Iran, it has a prevalence of 10.2%-11.9% and is one of the most common alleles (15-17). In our study, in all COVID-19 patients, it has a 36% prevalence, but in severe cases, along with DRB1*11, it was the most common allele present in 53.33% of cases. There has not been any reported connection between A*24 and susceptibility to SARS-CoV or SARS-CoV-2 (18), but it is reported as a risk factor for type 1 diabetes (19,20), systemic lupus erythematosus (21), myasthenia gravis (22), and Buerger's disease (23). Most of these diseases are categorized as an autoimmune disorders, and also it seems that COVID-19 shares a similar inflammatory immune response with autoinflammatory and autoimmune conditions, especially in severe or critical cases (24), so A*24 association with COVID-19 severity in our study may be related to its connections to hyperresponsiveness of the immune system. Our main limitation in this study was the small sample size due to high HLA-typing test prices, so we recommend doing similar studies with more COVID-19 cases to find more reliable correlations. Another suggestion is to use high-resolution HLA typing for future studies to find the precise HLA gene sequences associated with COVID-19. Finding HLAs alleles associated with COVID-19 susceptibility or resistibility can recommend certain HLA binder SARS-CoV-2 epitopes to facilitate better vaccine development (25). Surely future studies are needed to confirm such findings, but we hope it happens as soon as possible.

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