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Hla-A*32 is Associated with Severity of Covid-19 Patients

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Abstract

Objective: Recent advances have contributed to a better understanding of the shared and specific roles of HLA alleles in outcome of Covid-19 disease. We aimed to determine if a severe prognosis could be predicted.

Methods: Covid-19 patients were divided into severe (n=30) and non-severe (n=29) patient groups. All patients' demographic, clinical, laboratory, and treatment data were collected and analyzed. Class I/II HLA loci (A, B, C and DRB1, DQB1, DQA1) alleles were studied in patients and healthy controls (n=30), and outcomes of data were compared.

Results: From the demographic and clinical data, 28-day mortality, comorbidity, hypertension and coronary artery disease were found to be significantly higher in the severe group (SG) compared to the non-severe group (NSG). Of the parent-1 allele groups, A*26, A*32, B*41, C*14, C*16, DRB1*8, and DRB1*14 alleles were only present in the severe group and DQB1*4, B*27, B*52, and C*5 alleles were present in the severe and non-severe groups but not in controls. Also, while the presence of A*68, B*37, B*58, DRB1*16, DQB1*4, and C*14 alleles from parent-2 allele groups only in severe and non-severe groups may cause susceptibility to the disease, parent-2 allele groups A*1, DQB1*4, B*15 and B*54 were only present in healthy controls and may have a protective effect.

Conclusion: Since only the A*32 allele was detected in both parents only in the severe group, this allele may be associated with Covid-19 disease. These data suggest that some HLA alleles may be associated with the occurrence of Covid-19.

Keywords: 1- Coronavirus Disease 2019, 2- Human Leukocyte Antigens, 3- Immune Response, 4- Allele Frequency

Introduction

Coronavirus disease is a viral disease caused by the betacorona virus family virus SARS-CoV-2 (Covid-19). This virus, which is thought to originate from the animal market in Wuhan, China in 2019, has spread worldwide and infection can be fatal [1]. Coronaviruses are in the category of respiratory viruses that can cause a variety of infections from the common cold to Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) [2]. All three viruses from the coronavirus family (MERS, SARS, Covid-19) can cause infection that progresses to death by settling in the lower respiratory tract in elderly people and those with poor general health conditions [3].

Covid-19 disease can appear in four different clinical forms as asymptomatic, mild, moderate, and severe [3, 4]. Although the disease mostly manifests itself with pulmonary symptoms (cough, expectoration, shortness

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of breath, and $PaCO_2$ and SaO_2 reduction) [5], it also causes potentially lethal inflammation in extrapulmonary organs [2]. The presence of comorbidities (old age, lung, heart, or kidney disease, malignancy) together with the disease also affects the severity of the disease and causes an increase in the death rate [3-6]. According to the data from China, asymptomatic and mild patients constitute the majority of Covid-19 patients, while the moderate patient group constitutes 14% of the cases and the severe patient group constitutes 5% of the cases. However, while the death rate is 2.3% on average in the entire patient population with Covid-19, this rate rises to 49.0% in the severely ill group [3].

The first studies on Covid-19 disease showed that the immune system is active in the pathogenesis of the disease. IL-6, IL-7, TNFα, IL-10, G-CSF, MCP-1, MIP1α, and lymphokine and cytokine levels, mainly due to macrophage and granulocyte-derived cytokine storm [7], were found to be increased in the blood of patients, especially in severe Covid-19, and this increase is correlated with the severity of the disease [4, 6, 8, 9]. This cytokine storm causes damage to the lungs and causes clinical symptoms [10]. In order to understand the clinical course of Covid-19 disease, it is necessary to understand the relationship of the infection with the genetic structure of the patient and the patient's response to the infection [11]. The major-histocompatibility-complex antigen loci (HLA) system is localized in the short arm of the 6th chromosome, shows highly polymorphic features in the human genome, and plays a key role in the release of chemokines and cytokines by regulating the immune system in the presence of infection [12-15]. The HLA system includes three gene classes (Class I, II, III). Among them, Class-I HLA-A, B, C and Class-II HLA-DR, DQ, and DP haplotypes play a role in the formation of resistance, dissemination, and sensitivity against many infectious diseases by regulating various immunologic functions with antigen presentation [16, 17]. Allelic polymorphisms in the HLA system may also cause susceptibility to infection, affect clinical outcomes, and correlate with disease severity, such as SARS and MERS [10, 18-20].

Polymorphisms in the genes that create the immune-inflammatory response seen in Covid-19 infection or the genes in the HLA system play a key role in the inflammatory response, and the symptoms of Covid-19 disease and the differences in the clinical course of the disease (from asymptomatic, to mild-moderately symptomatic and severely affected patients requiring intensive care and respiratory support) [7]. Recently, many studies have been published investigating the association of different HLA alleles with Covid-19 disease to predict the severity or course of the disease [1, 11, 15, 19]. Of these [1] showed that the HLA system has an important role in the pathogenesis of Covid-19 disease. [21] Showed that HLA-DR molecules on circulating monocytes are reduced (downregulated) in patients with severe Covid-19 due to various patient-derived factors. [15] Found some HLA allele frequencies to be significantly higher in the patient group with Covid-19 compared to the control group. Yet another study [11] found that the affinity of Covid-19 peptides to bind to the HLA system differs between people according to geographic region. Since Covid-19 disease progresses with different clinical severity according to individuals and can progress to death in severe patients, we investigated the relationship between Class I and II HLA haplotype/allele groups and disease severity in Covid-19 patients in this study. We aimed to determine if a severe prognosis could be predicted.

Material and Methods

In this study, 59 patients with a positive Covid-19 RT-PCR test between August 2020 - August 2021 and who were followed up in Dışkapı Yıldırım Beyazıt Training and Research Hospital Infectious Diseases clinic due to Covid-19 infection were included. The samples were studied in the tissue typing laboratory of our hospital. This prospective study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Dışkapı Yıldırım Beyazıt Education and Research hospital (22 June 2020/No: 90/03). Informed consent was obtained from all individual participants included in the study. Among the patients with Covid-19 (PCR positive) included in the study, 30 patients who had the intensive care hospitalization criteria, which are dyspnea, respiratory frequency ≥30 breaths/min, oxygen saturation [SpO_2] ≤93%, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen [PaO_2/FiO_2] of 50% within 24 to 48 hours, respiratory failure, septic shock or multiorgan dysfunction or failure [3, 5, 22, 23], were considered as severe group (SG) and 29 patients who were followed only in the clinic were considered as non-severe group (NSG) were included in the study [24]. Demographics, comorbid diseases, clinical and laboratory findings of the study group were grouped by severity (Table-1). 30 people with negative Covid-19 PCR test results were included in the study as a healthy control (HC) group. Whole blood samples from the patient and control groups were taken into a hemogram tube and stored at 4-8 °C until the study day. Blood samples were collected in EDTA vacutainers. DNA isolation was performed from the blood taken at the Tissue Typing Laboratory of Dışkapı Yıldırım Beyazıt Training and Research Hospital Mevki Campus with a DNA isolation device. HLA tissue typing was performed using the sequence-specific oligonucleotide (SSOP) method with the Luminex-Fluoroanalyzer 2000 device using the isolated DNA. Luminex was used for validation and Match–it program was used for analysis. MHC Class-1 HLA (A, B, C) and Class-II HLA (DQA1, DQB1, DRB1) genes were examined in both parent haplotype allele groups (parent-1 and parent-2) and study groups (HC, SG and NSG) were compared.

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Statistical Analysis

GraphPad Prism 6.0 (GraphPad Software, CA, USA) software was used for statistical analysis. In descriptive analysis, mean±SD was used for the homogeneous parameters while the arithmetic median (Min.-Max.) was used for the non-homogeneous parameters. In intergroup comparisons, the Student's t-test or one-way ANOVA test, which is a parametric test, and the Mann-Whitney-U test or Kruskal–Wallis (Dunn's multiple comparisons or Wilcoxon rank), which is a non-parametric test, were used. Differences between the categorical variables were assessed using the Chi-square test. $p<0.05$ significance level was accepted. Number of units (n), percentage (%), mean, standard deviation, and median values are given as summary statistics.

Results

There was not a significant difference between the HC group (Female/Male, (13/17)) and the patient groups (SG and NSG) participating in the study in terms of gender distribution ($p=0.401$ and $p=0.240$). However, the difference between the ages was significant ($p<0.01$). This difference was due to the difference in the mean age between the groups when the mean age of SG was compared with HC (mean age 41.06±13.59 years) and NSG (SG vs HC and SG vs NSG, $p=0.001$; $p=0.001$). There was not a significant difference between the HC group and NSG in terms of mean age ($p>0.05$) (Table 1).

The demographic characteristics and comorbidity distribution of the patient group participating in the study, divided into severe and non-severe groups, are shown in Table 1. Among these results, mortality, hypertension (HT), and coronary artery disease (CAD) were found to be significantly higher in SG compared to NSG ($p<0.05$). However, there was no significant difference between the groups (SG vs NSG) in terms of smoking, duration of symptoms during hospitalization, diabetes (DM), chronic renal failure (CRF), cerebrovascular disease (CVD), congestive heart failure (CHF), and chronic lung disease (CLD) (Table 1). At the same time, haplotype and allele subgroups of Class-I HLA-A, B, C and Class-II HLA-DRB1, DQB1 and DQA1 were compared in both parental allele groups. No significant differences were found between the groups in terms of Class-I HLA-A, B, C and Class-II HLA-DRB1, DQB1 alleles in the HC, SG, and NSG groups ($p>0.05$) (Tables 2–7). However, although some allele groups were found to be high only in the HC group, either in the patient (SG and NSG) group, or only in SG, these differences were not significant between the groups.

Comparing the 2 parental allele groups for the HLA-A gene:

In the parent-1 allele group, A*01 and A*02 alleles were high in all three study groups, while A*26 and A*32 alleles were seen only in SG. In the parent-2 allele group, the A*24 and A*26 alleles was higher in the HC group but this was not significant compared to SG and NSG, the A*11 allele was higher in the SG compared to the NSG and HC groups, the A*68 allele was higher in SG group compared to the NSG group but was not seen in HC group. A*25 and A*32 alleles were only seen in SG group (Table 2).

For the HLA-B gene, when the 2 parental allele groups are compared:

In the parent-1 allele group, the HLA-B*8, B*35 and B*38 alleles were high in all three study groups, while the

Table 1: Distribution of Descriptive Characteristics of the Patient Group

	Total (n=59)	Non-Severe (n=29)	Severe (n=30)	
	n (%)	n (%)	n (%)	P
Gender (Female /Male)	30 (50.8) / 29 (49.2)	17 (58.6) / 12 (41.4)	13 (43.3) / 17 (56.7)	*0.24*
Age (Mean±SD)	56.31±15.65	49.45±13.04	62.93±15.28	*0.01*
Smoking (No/Yes)	41 (69.5) / 18 (30.5)	23 (79.3) / 6 (20.7)	18 (60.0) / 12 (40.0)	*0.107*
Symptom duration at admission (Mean±SD)	4.45±3.19	4.03±2.86	4.86±3.48	*0.238*
Malignancy (absent/present)	55 (93.2) / 4 (6.8)	27 (93.1) / 2 (6.9)	28 (93.3) / 2 (6.7)	*1*
28-day mortality (No/Yes)	51 (86.4) / 8 (13.6)	29 (100) / 0 (0)	22 (73.3) / 8 (26.7)	*0.005*
Comorbidity (No/Yes)	21 (35.6) / 38 (64.4)	16 (55.2) / 13 (44.8)	5 (16.7) / 25 (83.3)	*0.002*
DM (No/Yes)	40 (67.8) / 19 (32.2)	22 (75.9) / 7 (24.1)	18 (60.0) / 12 (40.0)	*0.192*
HT (No/Yes)	35 (59.3) / 24 (40.7)	23 (79.3) / 6 (20.7)	12 (40.0) / 18 (60.0)	*0.002*
CRF (No/Yes)	56 (94.9) / 3 (5.1)	29 (100) / 0 (0)	27 (90.0) / 3 (10.0)	*0.237*
CAD (No/Yes)	48 (81.4) / 11 (18.6)	27 (93.1) / 2 (6.9)	21 (70.0) / 9 (30.0)	*0.023*
CVD (No/Yes)	57 (96.6) / 2 (3.4)	29 (100) / 0 (0)	28 (93.3) / 2 (6.7)	*0.492*
CHF (No/Yes)	56 (94.9) / 3 (5.1)	28 (96.6) / 1 (3.4)	28 (93.3) / 2 (6.7)	*1*
CLD (No/Yes)	47 (79.7) / 12 (20.3)	25 (86.2) / 4 (13.8)	22 (73.3) / 8 (26.7)	*0.219*

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Table 2: Distribution of Parental HLA-A Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-A Parent 1	*Median (Q1-Q3)*	2 (1-3)	2 (1-3)	2 (2-11)	[a]***0.608***
HLA-A	**1**	9	8	4	
Parent 1*; n*	**2**	9	9	15	
	3	5	6	1	
	11	2	1	3	
	23	1	1	1	
	24	4	4	4	
	26	0	0	1	
	32	0	0	1	
HLA-A Parent 2	*Median (Q1-Q3)*	24 (11-26)	24 (3-31)	26 (11-32)	[a]***0.119***
HLA-A	**1**	3	0	0	
Parent 2*; n*	**2**	3	5	2	
	3	1	4	0	
	11	2	3	6	
	23	1	0	2	
	24	10	4	3	
	25	0	0	1	
	26	6	3	4	
	29	2	2	1	
	30	1	0	1	
	31	0	1	0	
	32	0	0	3	
	33	1	3	0	
	66	0	1	1	
	68	0	2	6	
	69	0	1	0	

Table 3: Distribution of Parental HLA-B Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-B Parent 1	*Median (Q1-Q3)*	35 (15-40)	35 (15-38)	38 (35-41)	[a]***0.176***
HLA-B	**7**	2	1	0	
Parent 1*; n*	**8**	2	3	4	
	13	2	2	0	
	14	1	1	0	
	15	3	4	0	
	18	4	1	0	
	27	0	1	1	
	35	4	6	8	
	37	2	1	0	
	38	2	2	6	
	39	0	1	0	
	40	2	0	3	

	41	0	0	2	
	44	2	1	3	
	49	1	1	0	
	50	1	1	1	
	51	2	0	1	
	52	0	1	1	
	55	0	2	0	
HLA-B Parent 2	*Median (Q1-Q3)*	50.5 (38-52)	51 (40-51)	51 (49-52)	[a]***0.326***
HLA-B	**14**	0	1	0	
Parent 2*; n*	**15**	2	0	0	
	35	5	4	1	
	37	0	1	1	
	38	2	1	1	
	40	1	2	1	
	41	2	2	0	
	44	1	2	2	
	45	0	0	1	
	49	1	0	3	
	50	1	1	1	
	51	4	8	10	
	52	8	1	2	
	54	1	0	0	
	55	1	2	4	
	57	1	2	2	
	58	0	2	1	

B*27 and B*52 alleles were detected only in SG and NSG. B*41 allele was detected only in SG. In the parent-2 allele group, the HLA-B*51 allele was higher in the SG and NSG group compared to the HC group, and the B*52 allele was higher in the HC group compared to SG and NSG. B*54 allele was detected only in HC, and B*37 and B*58 alleles were detected only in SG and NSG. B*45 allele was detected only in SG (Table 3).

In terms of HLA-C gene, when 2 parental allele groups are compared:

In the parent-1 allele group, the HLA-C*4, C*6, and C*7 alleles were found to be high in all three groups, however the C*12 allele was found to be high in HC groups compared to the other groups (SG and NSG). C*14 and C*16 alleles were detected only in SG, and C*5 allele was detected only SG and NSG. In the parent-2 allele group, the HLA-C*7, C*12, and C*15 alleles were high in all three study groups, while the C*14 allele was detected only in SG and NSG (Table 4).

When the 2 parental allele groups are compared for the HLA-DRB1 gene:

In the parent-1 allele group, HLA-DRB1*3 allele was high in HC, DRB1*4 allele was high in SG and NSG (especially NSG), and DRB1*11 allele was high in SG according to HC and NSG, while DRB1*8 and DRB1*14 alleles were found only in SG. In the parent-2 allele group, the HLA-DRB1*11 and DRB1*13 alleles were higher in all three groups, and the DRB1*15 allele was higher in the HC group compared to the other groups. DRB1*16 allele was found only SG and NSG (Table-5).

When the 2 parental allele groups are compared for the HLA-DQB1 gene:

While HLA-DQB1*2 allele was high in HC in parent-1 allele group, DQB1*3 and DQB1*5 alleles were high in all three study groups, DQB1*4 allele was detected only in SG and NSG. In the parent-2 allele group, the HLA-DQB1*3, DQB1*5, and DQB1*6 alleles were high in all three groups, while the DQB1*4 allele was seen only in HC (Table 6).

In terms of the HLA-DQA1 gene, when the 2 parental allele groups are compared:

In the parent-1 allele group, especially the HLA-DQA1*1 allele and DQA1*3 and DQA1*5 alleles were found to be high in all three groups, while in the parent-2 allele group, the HLA-DQA1*1 and DQA1*5 alleles were found to be high in all three groups (Table-7).

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Table 4: Distribution of Parental HLA-C Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-C Parent 1	*Median (Q1-Q3)*	5 (4-7)	5 (3-7)	4.5 (4-7)	[a]***0.773***
HLA-C	**1**	1	4	2	
Parent 1; *n*	**2**	4	2	1	
	3	1	2	4	
	4	9	6	8	
	5	0	1	2	
	6	5	6	4	
	7	5	7	5	
	12	5	1	2	
	14	0	0	1	
	16	0	0	1	
HLA-C Parent 2	*Median (Q1-Q3)*	12 (7-12)	12 (7-14)	12 (7-15)	[a]***0.458***
HLA-C	**3**	1	0	1	
Parent 2; *n*	**4**	1	3	1	
	6	1	2	2	
	7	6	4	5	
	8	2	2	0	

Table 5: Distribution of Parental HLA-DRB1 Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-DRB1 Parent 1	*Median (Q1-Q3)*	7 (3-11)	4 (4-11)	9 (4-11)	[a]***0.620***
HLA-DRB1	**1**	2	3	4	
Parent 1; *n*	**3**	7	4	3	
	4	5	10	6	
	7	4	3	1	
	8	0	0	1	
	9	0	1	0	
	10	2	0	1	
	11	5	4	8	
	13	4	2	3	
	14	0	0	2	
	15	1	2	1	
HLA-DRB1 Parent 2	*Median (Q1-Q3)*	13 (11-14)	13 (11-14)	13 (11-14)	[a]***0.939***
HLA-DRB1	**3**	0	1	0	
Parent 2; *n*	**4**	3	2	4	
	7	2	0	1	
	8	1	2	2	
	11	6	7	7	
	13	6	6	5	
	14	5	4	4	
	15	7	3	3	
	16	0	4	4	

Table 6: Distribution of Parental HLA-DQB1 Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-DQB1 Parent 1	*Median (Q1-Q3)*	3 (2-3)	3 (3-3)	3 (3-5)	[a]***0.254***
	2	11	6	4	
	3	12	17	16	
	4	0	1	2	
	5	4	4	6	
	6	3	1	2	
HLA-DQB1 Parent 2	*Median (Q1-Q3)*	5 (3-6)	5 (3-6)	5 (3-6)	[a]***0.532***
	3	10	11	13	
	4	1	0	0	
	5	5	8	8	
	6	14	10	9	

Table 7: Distribution of Parental HLA-DQA1 Values by Case Severity

		Case Severity			
		Control	Mild	Severe	P
HLA-DQB1 Parent 1	*Median (Q1-Q3)*	3 (2-3)	3 (3-3)	3 (3-5)	[a]***0.254***
	2	11	6	4	
	3	12	17	16	
	4	0	1	2	
	5	4	4	6	
	6	3	1	2	
HLA-DQB1 Parent 2	*Median (Q1-Q3)*	5 (3-6)	5 (3-6)	5 (3-6)	[a]***0.532***
	3	10	11	13	
	4	1	0	0	
	5	5	8	8	
	6	14	10	9	

Discussion

With this study, we aimed to investigate the possibility of using genetic findings with laboratory parameters to predict the severity of Covid-19 and help in patient follow-up and risk assessment to reduce disease morbidity and mortality, since the disease is severe in the presence of advanced age and comorbidities. In our study, advanced age and comorbidities such as hypertension, diabetes, and coronary heart disease were associated with the severity of the disease and death due to the disease in accordance with previous studies [4-6, 24-26] in Covid-19 patients (Table 1). According to studies, the mortality rate (CFR) (case fatality rate) in all cases was found to be 2.3% [3]. On the other hand, while death was not observed in children under 9 years of age, the mortality rate increased in correlation with comorbidities and advanced age. CFR was elevated among those with preexisting comorbid conditions: 10.5% for cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer [3].

The HLA system plays a key role in the regulation of the immune system in humans [11]. For this reason, although the HLA allele system in Covid-19 patients has been studied by many researchers, there are very different results in this regard. In some studies, low or high HLA alleles in the study groups were found to be associated with the disease or disease severity [15, 19, 25, 27, 28]. However, in some studies, low or high alleles between the groups were not significant and not associated with the disease or disease severity [25, 29]. This diversity of studies is due to the large polymorphic feature of the HLA system and regional genetic differences or the small number of patients in the study groups [1, 26, 30].

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According to the study by [26], which includes the relationship between Class-I HLA (AC) and Class-II HLA (DPB1, DQA1, DQB1, and DRB1) genes in Covid-19 patients and the severity of the disease, especially HLA-A*11:01, B*. They interpreted that the 51:01 and C*14:02 alleles were significantly higher in severe and critical patients compared to mild and moderate patients, as Class-I HLA alleles were more correlated with disease severity than Class-II HLA alleles. [15, 27] Support the conclusion that Class-I HLA (A-C) alleles are more correlated with disease severity in Covid patients, as in our study results. While [15] found HLA-C*07:29, B*15:27 alleles to be significantly higher in the patient group compared to the control group, [27] found HLA-A*11:01, C*12:02 and B*52:01 alleles from Class-I allele groups to be higher in patients with severe Covid-19 compared to patients with mild Covid-19. We also found in our study that Class-I HLA A*26, A*32, B*27, B*41, B*52, C*5, C*14, and C*16 alleles from parent-1 allele groups and Class-I HLA alleles A*11, A*68, A*25, A*38, B*37, B*58, B*45, B*51 and C*14 alleles from parent-2 allele groups were significantly higher in the patient groups (SG and NSG). (Table 2-4).

In addition, [15] determined that DRB1*14:04, DRB1*01:01, and DQA1*01:01 alleles were higher in the severe group compared to the mild group as a risk allele for the severity of the disease, while DPB1*03:01 and DRB1*12*01 alleles were higher in the mild group compared to the severe group. Therefore, they evaluated the effect of these alleles (DPB1*03:01 and DRB1*12*01) to show their protective effect against the disease. We evaluated A*26, A*32, B*41, C*14, C*16, DRB1*8 and DRB1*14 alleles from parent-1 detected only in SG and DQB1*4, B*27, B*52, C*5 and A*68, B*37, B*58, C*14, DRB1*16 alleles from parent-1 and parent-2 alleles detected only SG and NSG, respectively, as risk alleles for Covid-19 disease, and A*1, DQB1*4, B*15, and B*54 alleles from parent-2 allele groups detected only in HC group as protective effective alleles. The fact that the risk and protective alleles for Covid-19 disease we found differ from the results of the study conducted by Wang et al. [15] study may be due to the small size of our study group or genetic polymorphisms due to regional differences [1-15]. Again, [15] found the HLA-B*46:01 allele to be higher in the mild patient group than in the severe group, unlike the study results of Nguyen et al., but this difference was not significant. According to the study by [7] with HLA haplotypes in Italian patients with Covid-19, HLA-A*01:01, B*08:01, C*07:01, and DRB1*03:01 alleles were significantly positively correlated with the disease. These alleles are associated with susceptibility to Covid-19, while HLA-A*02:01, B*18:01, C*07:01, and DRB1*11:04 alleles were significantly inversely correlated with the disease. They thought that there might be protective alleles for Covid-19. Therefore, both groups of alleles were associated with the incidence and mortality of Covid-19 in Covid-19 patients. In addition, [31] found a significant negative correlation between the presence of the HLA-DRB1*01 allele in the Mexican population and the death rate due to Covid-19 disease according to their broad-based data analysis in Mexico.

In our study, when allele subtypes were not taken into account, we found the frequencies of alleles found by [7] to be high in all three study groups (HC, SG, NSG). However, the frequency differences between the groups were not significant. In addition, according to our study results, although there was not a significant difference between the groups, the alleles that could have susceptibility and protective effects in Covid-19 patients were completely different from the alleles found by [7]. According to our study results, alleles seen only in the healthy control group were protective alleles, while alleles seen only in the sick or severely ill group were alleles that could cause sensitization. [30] found a positive correlation between Covid-19 disease and especially HLA-B*44 and C*01 alleles and a negative correlation between B*14, B*18, and B*49 alleles in relation to the spread of Covid-19 disease in Italy. Although the study by [30] is partially similar to our study in terms of alleles detected and their results, both in terms of the frequency of alleles studied and especially from parent-1 allele groups A*26, A*32, B*41, C*14, C*16. and DRB1*14 alleles differ with our study results that A*68 and B*58 alleles from the parent-2 allele group may cause susceptibility to Covid-19. In addition, study results conducted by Correale et al. [30] were inconsistent with the study by Novelli et al. in Italy [19]. According to the study by Novelli et al. [19], while HLA-DRB1*15:01, DQB1*06:02, and B*27:07 alleles cause susceptibility for Covid-19 disease in the patient group with Covid-19, there was no significant difference between the groups in terms of the HLA-B*58:01 allele. C*06:02 and DRB1*07:01 alleles were negatively correlated with Covid-19 disease [19]. In addition, according to our results, HLA-DRB1*15:01, DQB1*06:02, and B*27:07 alleles were high in our study group as HC, SG, NSG, but the difference between the groups was not significant, whereas the results were significant and high in the patient group in the study by Novelli et al. [19]. The reason for this significant difference could depend on geographical and regional differences, which is also mentioned in the studies done by Nguyen et al. [1] and Correale et al. [30].

According to the results of the study done by Nguyen et al. [1], A*02:02, B*15:03 [11] and C*12:03 from the HLA-A, B and C alleles were very good at binding the Covid-19 peptides, although A*25:01, B*46:01 [11] and C*01:02 alleles were poor at binding the Covid-19 peptides. The conclusion that Covid-19 peptides can be presented mostly by class I HLA alleles was found to be consistent with the results from Wang et al. [26] and our results. In our study, we also found higher Class-I HLA allele frequencies in Covid-19 patients.

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In addition, Nguyen et al. [1] defined the presence of the HLA-B*46:01 allele in the patient population as a possible risk factor for disease severity [32] and overexpression of the HLA-B*15:03 allele as a protective factor [11, 33]. Although these results were similar in terms of the B*15 allele as a protective factor in our study, they were different in terms of the B*46 allele because we could not detect B*46 allele in either parental allele group. This is likely due to the small size of our study population.

According to the study conducted by Iturrieta-Zuazo et al. [24], the affinity of Class I HLA (A, B, C) genes to bind Covid-19 peptides in Covid-19 patients was not correlated with clinical severity. Compared with the light or tight binding of the Covid-19 peptides to the HLA alleles, it was found that there was a significantly higher light and tight binding affinity in mild patients (HLA-C, Mild vs Moderate, excluded) compared to moderate and severe patients. According to the results of the study, the relationship of peptide presentation and binding was not associated with disease severity, unlike the results of Nguyen et al [1]. The distribution of alleles between groups was not different, nor was the distribution between groups according to supertype [24]. This result was different according to study results shown by Wang et al. [26] and Nguyen et al. [1]. According to Wang et al. [26], Class I genes are more important than Class II genes in terms of the severity of the disease in Covid-19 patients. According to Iturrieta-Zuazo et al. [24], the higher detection of Class I HLA genes in the mild group may be due to the high heterozygous distribution of alleles in the mild group. Because of the combination of polymorphisms in the HLA system, heterozygous individuals may be more effective in presenting environmental pathogens to immune system cells than homozygous individuals [34]. Lorente et al. [25], in their study of 72 patients (10 non-survivors and 62 survivors) with Covid-19 and a healthy control group, HLA-A*32 allele were high in the healthy control group, and HLA-B*39 and C*16 alleles were be high in the patient group. However, this difference in frequency between the groups was not significant. In addition, in their analysis dividing the severe patient group into two, as survivors and non-survivors, they found HLA-A*11, C*01, and DQB1*04 alleles to be significantly higher in the non-survivor group [25]. In our study, we detected the HLA-A*32 allele only in SG and the DQB1*4 allele only in the patient and HC group, and our results in this respect differed from the results of Lorente et al. [25]. Despite the similarities and differences in the results of all these studies, there is a close connection between the Covid-19 disease and the patient's genetic structure (HLA allele system).

Conclusion

In conclusion, the fact that we detected some alleles only in the HC group and some only in SG or SG and NSG in our study suggests that the HLA system may have a role in the clinical course of Covid-19 disease. However, this clinical difference with other studies in terms of alleles may be due to regional differences in allele frequency, as well as the difference in allele distribution due to various factors such as homozygous or heterozygous alleles and may explain this difference. Among HLA class I, HLA-B is the most polymorphic class Class-1 gene (4,077 alleles identified), while HLA-A gene contains 3,285 alleles and HLA-C gene contains 2,801 alleles [35]. For this, large study groups are needed, together with large gene database studies, in order to understand the behavior pattern of Covid-19 disease and to predict the clinical course. Therefore, the low number of patients participating in our study may have limited our results.

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Disclosure of potential conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Research involving human participants and/or animals

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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