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Research article

HLA-B*15 predicts survival in Egyptian patients with COVID-19

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ABSTRACT

Genetic differences among individuals could affect the clinical presentations and outcomes of COVID-19. Human Leukocyte Antigens are associated with COVID-19 susceptibility, severity, and prognosis. This study aimed to identify HLA-B and -C genotypes among 69 Egyptian patients with COVID-19 and correlate them with disease outcomes and other clinical and laboratory data. HLA-B and -C typing was performed using Luminex-based HLA typing kits. Forty patients (58%) had severe COVID-19; 55% of these patients died, without reported mortality in the moderate group. The alleles associated with severe COVID-19 were HLA-B*41, -B*42, -C*16, and -C*17, whereas HLA-B*15, -C*7, and -C*12 were significantly associated with protection against mortality. Regression analysis showed that HLA-B*15 was the only allele associated with predicted protection against mortality, where the likelihood of survival increased with HLA-B*15 (P < 0.001). Patient survival was less likely to occur with higher total leukocytic count, ferritin, and creatinine levels. This study provides interesting insights into the association between HLA class I alleles and protection from or severity of COVID-19 through immune response modulation. This is the first study to investigate this relationship in Egyptian patients. More studies are needed to understand how HLA class I alleles interact and affect Cytotoxic T lymphocytes and natural killer cell function.

1. Introduction

Coronavirus disease 2019 (COVID-19) has been recognized as a pandemic by the World Health Organization [1]. The disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with a wide range of symptoms, which could be mild, moderate, or severe leading to multiorgan failure or death [2,3]. Most patients with COVID-19 have a good prognosis. However, critical symptoms develop in about 5% to 10% of them, which may be fatal in about 6% of the cases [4-6]. Several factors, including older age, male gender, and comorbidities, are associated with the development of severe complications and death [7,8].

Several questions about the role of the genetic origin of the variability of the immune response to SARS-CoV-2 continue to emerge. COVID-19 complications are usually the sequel of excessive and dysregulated immune responses [9], where severe COVID-19 is thought to be the result of the so-called "cytokine storm" and hypercytokinemia, which plays a key role in disease progression [5].

Human leukocyte antigens (HLA) are encoded by genes within the HLA super-locus, located within the 6p21 chromosomal region. This locus contains genes that code for the six classical HLA proteins and many other proteins that play important roles in the regulation of the immunity, and other cellular and molecular processes [10]. The HLA proteins play a key role in the ability of the immune system to recognize and develop immunity against microbes. Both HLA classes (I and II) present peptides derived from foreign organisms on the cell surface to be recognized by T cells. Immunogenic peptides presented with HLA class I on the surface of nucleated cells are recognized by cytotoxic CD8^{+} T cells, whereas peptides bound by HLA class II on the surface of antigen-presenting cells activate CD4^{+} T cells, coordinate and regulate the function of effector cells of the immune system [11]. HLA alleles play an essential role in the antigen presentation of different viruses. Previous studies have shown that different alleles are associated with the

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variable susceptibility to and severity and prognosis of the viral infection [12,13].

The SARS-CoV-2 gene sequence has significant similarity to SARS-CoV, although the two viruses do have distinct variations [14]. HLA was studied in a previous work during the SARS outbreak in 2003, where the HLA-B*46:01 allele was associated with disease severity in a group of Taiwanese patients [15]. In another study in Hong Kong Chinese patients, HLA-B*07:03 and HLA-DRB1*03:01 alleles were associated with increased susceptibility to SARS infection [16].

Although many risk factors have been identified for severe COVID-19, discovering the relationships between genetic differences and different clinical presentations and mortality still need to be explored [17]. The host immune response could be linked to disease severity and dysfunctional immune response, which may lead to the severe or progressive course of the disease [18]. HLA alleles play an important role in the immune response against infectious diseases. Many studies highlighted the existence of significant associations between some HLA alleles and the susceptibility, severity, or clearance of infectious diseases, including malaria, tuberculosis, leprosy, HIV, and hepatitis [18-20].

Some authors hypothesized that different HLA alleles might also be associated with variable individual susceptibility to and prognosis of SARS-CoV-2 infection, similar to what was reported for the two previous members of the coronaviruses family responsible for SARS and the Middle East respiratory syndrome [12-14], and one of the early studies that evaluated the correlation between HLA alleles and COVID-19 prevalence was published in June 2020. Correale et al. showed that HLA-B*44 and HLA-C*01 were independently associated with COVID-19 spreading in Italy [12]. Based on these results, this study aimed to identify HLA-B and HLA-C genotypes among a group of Egyptian patients with COVID-19 and correlate these genotypes with disease outcomes and other clinical and laboratory data, especially those known to have prognostic significance.

2. Material and methods

2.1. Patients

This study included 69 COVID-19 hospitalized patients at Assiut University Hospitals. COVID-19 diagnosis was confirmed using upper respiratory specimens (nasopharyngeal swabs) analyzed using real-time polymerase chain reaction (PCR) for SARS-CoV-2. The study was approved by the Ethical Committee of the South Egypt Cancer Institute, Assiut University, and all patients signed informed consent before inclusion in the study. Clinical, radiological, and laboratory data were collected from patients' files. Patients were categorized into mild, moderate, and severe/critical categories according to Wu et al. [17], and the outcome was recorded. Samples and data were collected between July and September 2020.

Mild disease was defined as the presence of clinical symptoms and no changes in computed tomography (CT) chest. Moderate cases included all cases with respiratory symptoms associated with changes in CT. Severe cases were defined by the presence of the following criteria: (1) respiratory distress with respiratory rate (RR) $\geq$ 30/min, (2) resting blood oxygen saturation $\leq$ 93%, or (3) partial pressure of arterial blood oxygen (PaO_2)/oxygen concentration (FiO_2) $\leq$ 300 mmHg. Critically ill cases included all severe cases that deteriorated due to (1) respiratory failure and needed mechanical ventilation, (2) shock, and (3) other organ failure needing intensive care unit (ICU) monitoring treatment.

2.2. HLA-B and HLA-C typing using a Luminex-based HLA typing kits

Luminex-based HLA typing kits apply Luminex® technology to reverse the sequence-specific oligonucleotide (SSO) DNA typing

method. First, 2 ml EDTA peripheral venous blood was collected from patients under complete aseptic conditions. Genomic DNA was isolated from blood samples using the QIAamp DNA mini extraction kit (Qiagen, Hilden, Germany) that provides silica membrane-based DNA purification.

HLA-B and HLA-C typing was performed using the LAB Type® SSO typing kits (LAB Type SSO Class I B Locus Cat. RSSO1B, Lot 021, and LAB Type SSO Class I C locus, Cat. RSSO1C, Lot 014, One Lambda, Canoga Park, California) that provide SSO probes immobilized on a set of fluorescently labeled microspheres for HLA allele identification in amplified genomic DNA samples through a controlled DNA/DNA hybridization reaction, followed by flow analysis using LABScan™ 100.

Target DNA was PCR amplified using group-specific biotin-labeled primers. PCR amplification was programmed at 96 °C for 3 min, followed by five cycles of 96 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s, 30 cycles of 96 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s, and extension at 72 °C for 10 min. DNA amplification was followed by gel electrophoretic analysis to check the amplification of specific exons of each locus.

PCR products generated using each locus-specific set of primers were then denatured and allowed to rehybridize to cDNA probes conjugated to fluorescently coded microspheres. The biotinylated PCR product bound to the microsphere was labeled with streptavidin conjugated with R-phycoerythrin. The hybridized products were analyzed using a flow analyzer running LABScan™ 100 xPO-NENT (One Lambda). The fluorescence intensity in each microsphere was identified by HLA Fusion™ version 4.5 (One Lambda), where a low resolution was applied for HLA data analysis and the assignment of HLA typing based on the reaction pattern was compared to patterns associated with published HLA gene sequences. The allele nomenclature included the HLA alleles listed in the July 2009 update of the IMGT/HLA database release 2.26.0 [21].

2.3. Statistical analysis

Statistical analysis was performed by Minitab 17.1.0.0 for Windows (Minitab, Inc., 2013, State College, PA, USA). The normality of data was examined using the Shapiro-Wilk test. Continuous data were presented as the mean and standard deviation (SD) or median and interquartile range (IQR), whereas categorical data were presented as the number (percentage). A comparison between two groups of continuous data was performed using an independent t-test or Mann-Whitney test, and a comparison between two or more groups of categorical data was done using the χ^2 test. The χ^2 goodness-of-fit tests were used to examine the association between different HLA-B and HLA-C alleles with COVID-19 severity and survival. PC-ORD for Windows (version 5) was used for two-way hierarchical cluster analysis using Sorensen methods for distance and beta (-0.025) for group linkage. Multiple logistic regression models with stepwise elimination technique and adjustment for age, sex, comorbidity, use of continuous positive airway pressure (CPAP) and steroids in treatment, and laboratory data of patients were performed to find any possible predictors of survival in COVID-19 patients. All tests were two-sided. P < 0.05 was considered significant.

3. Results

3.1. General characteristics of COVID-19 patients and frequency of HLA-B and HLA-C alleles

In Table 1, both moderate and severe cases were matched to age, sex, smoking habits, and comorbidity, except for renal disease, where four cases with renal failure had severe COVID-19 pneumo-

Table 1
Clinical and laboratory characteristics of COVID-19 patients (n = 69).

Factors	Moderate (n = 29)		Severe (n = 40)		P
	Median	IQR	Median	IQR	
Age	62	(53.5–68)	62	(54.5–67)	0.93$^\$$
Sex	n	%	n	%	
Female	15	51.72	23	57.5	0.63#
Male	14	48.28	17	42.5	
Smoking	n	%	n	%	
Nonsmoker	19	65.52	33	82.5	0.1#
Smoker	10	34.48	7	17.5	
Comorbidities (yes)	n	%	n	%	
Diabetes mellitus	5	17.24	15	37.5	0.06#
IHD	1	3.45	3	7.5	0.6#
Hypertension	10	34.48	14	35	0.96#
Renal disease	0	0	4	10	**0.03#**
Clinical presentation(s) (yes)	n	%	n	%	
Cough	28	96.55	40	100	0.42#
Dyspnea	25	86.21	38	95	0.23#
Fever	21	72.41	32	80	0.46#
Bony aches	5	17.24	4	10	0.47#
Disturbed conscious level	0	0	4	10	**0.03#**
Fatigue	4	13.79	5	12.5	0.85#
	Median/mean	IQR/SD	Median/mean	IQR/SD	
Systolic blood pressure (mm Hg)	130	(110–130)	120	(110–140)	0.93$^\$$
Diastolic blood pressure (mm Hg)	80	(80–90)	80	(70–90)	0.99$^\$$
Temperature	38	(37.65–39)	38	(38–39)	0.65$^\$$
Pulse (bpm)	87.55	7.54	88.45	9.77	0.68$^\$$
RR (cycle/min)	28	(25–29)	36	(33–38)	**<0.001$^\$$**
O$_2$ saturation (%)	93	(93–94)	88.5	(82.25–90)	**<0.001$^\$$**
Laboratory findings	Median/mean	IQR/SD	Median/mean	IQR/SD	
TLC × 10^9	6.6	(4.35–10.8)	8.9	(6.6–13.86)	**0.02$^\$$**
Lymphocyte (%)	15.38	(9–25.76)	10.15	(8.8–16)	**0.05$^\$$**
Hemoglobin (mg/dL)	12.918	1.744	12.05	1.559	**0.03$^\$$***
Platelet × 10^9	274	(178.5–311.5)	247	(194.5–325)	0.96$^\$$
ALT (IU/L)	29	(27–35.5)	34	(29.25–39)	**0.04$^\$$**
AST (IU/L)	34	(32–40)	41	(39–48.75)	**0.002$^\$$**
CRP (mg/L)	65	(53.5–70.5)	96.5	(79–105)	**<0.001$^\$$**
Ferritin (mic/L)	442	(387.5–517.5)	727.5	(431–942.75)	**<0.001$^\$$**
Creatinine (mg/dL)	0.8	(0.6–0.9)	1.2	(0.82–1.9)	**<0.001$^\$$**
Na	130	(129–131.75)	129	(128–130)	**0.01$^\$$**
K	3.8	(3.7–4.1)	3.9	(3.7–4.17)	0.96$^\$$
Admission zone	n	%	n	%	
ICU	0	0	21	52.5	**<0.001#**
Ward	29	100	19	47.5	
O$_2$ supplementation device	n	%	n	%	
Face mask	29	100	17	42.5	**<0.001#**
CPAP	0	0	10	25	
Mechanical ventilation	0	0	13	32.5	
Steroid use (yes)	17	58.62	30	75	0.15#
Anticoagulation therapy	n	%	n	%	
Heparin	0	0	5	12.5	
Clexane (therapeutic does)	7	24.14	8	20	0.14#
Clexane (prophylactic does)	22	75.86	27	67.5	
Chloroquine (yes)	2	6.9	4	10	0.67#
Vitamins (yes)	22	75.86	32	80	0.68#
Outcome	n	%	n	%	
Died	0	0	22	55	**<0.001#**
Survived	29	100	18	45	
	Median	IQR	Median	IQR	
Length of hospital stay (days)	10	(9–11)	5	(4–8.75)	**<0.001$^\$$**

Continuous data are the mean (SD) or median (IQR), and categorical data are the n (%).
CRP: C-reactive protein, ICU: Intensive care unit, CPAP: Continues positive pressure ventilation.
  # χ^2 test, $^\$$Mann-Whitney test, $^\$$*Independent *t*-test. P < 0.05 is considered significant.

nia (P = 0.03). The symptoms and signs significantly associated with severe COVID-19 were *disturbed conscious level* (P = 0.03), *increased RR* (P < 0.001), and *decreased oxygen saturation* (P < 0.001). Regarding laboratory findings, the severe group was characterized by an elevated total leukocytic count (TLC; P = 0.02), lower percentage of lymphocytes (P = 0.05), and elevated serum aspartate aminotransferase (AST; P = 0.04), alanine aminotransferase (ALT; P = 0.002), and creatinine (P < 0.001). The levels of inflamma-

tory markers, C-reactive protein (CRP) and ferritin were significantly higher in the severe group (P < 0.001 for both). More than half of the severe cases were admitted to the ICU, with significant demand for mechanical ventilation and CPAP for oxygen supply (P < 0.001 for both). The length of hospital stay was significantly prolonged in moderate COVID-19 cases (median of 10 days) compared to a median of 5 days in the severe group (P < 0.001). Although hospital stay was longer in the moderate group, all patients in this category recovered and were discharged. In contrast, the mortality rate in the severe group was 55%. The frequency of each HLA-B allele and genotype are presented Supplementary Tables 1 and 2 and that of each HLA-C allele and genotype are presented in Supplementary Tables 3 and 4.

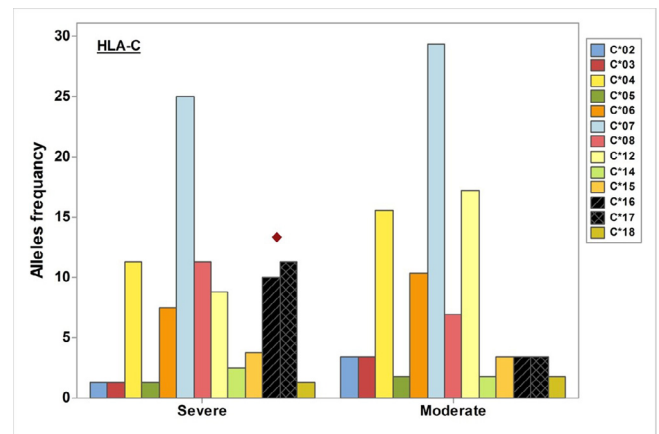### 3.2. Frequency of HLA-B and HLA-C alleles in COVID-19 patients and correlation with severity and mortality

Fig. 1 shows the frequency of each allele in correlation with the severity of the disease. HLA-B*41, HLA-B*42, HLA-C*16, and HLA-C*17 were significantly associated with severe COVID-19 pneumonia (P = 0.05, 0.01, 0.05, and 0.03, respectively; Supplementary Tables 5 and 6).

3.3. Predictors of survival in COVID-19 patients

In Fig. 2 and Supplementary Tables 7 and 8, HLA-B*15 was significantly associated with protection against mortality (P = 0.02), same for HLA-C*07 and HLA-C*12 (P = 0.001 and 0.008, respectively). Regression analysis models were used to predict the factors associated with survival, with adjustment for age, gender, comorbidity, use of CPAP and steroids in treatment, and laboratory data of patients. In Table 2, the significant predictors of survival were HLA-B*15, TLC, ferritin and creatinine levels, and the presence of ischemic heart disease (IHD). The likelihood of survival increased up to 1351-fold with the presence of the HLA-B*15 allele (odds ratio (OR) = 1351.06; P < 0.001). The survival of COVID-19 patients was less likely to occur with elevated TLC, ferritin and creatinine levels (OR = 0.56, 0.98, and 0.36 respectively; P < 0.001 for all), and the presence of IHD (OR = 0.01; P = 0.03).

### 3.4. Association between HLA-B and HLA-C haplotypes

A cluster analysis was performed using all HLA-B and HLA-C alleles to find any association between HLA-B and HLA-C alleles
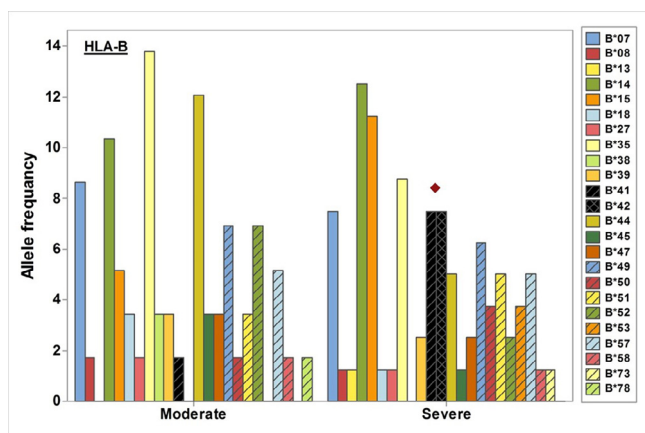
Fig. 1 (continued)
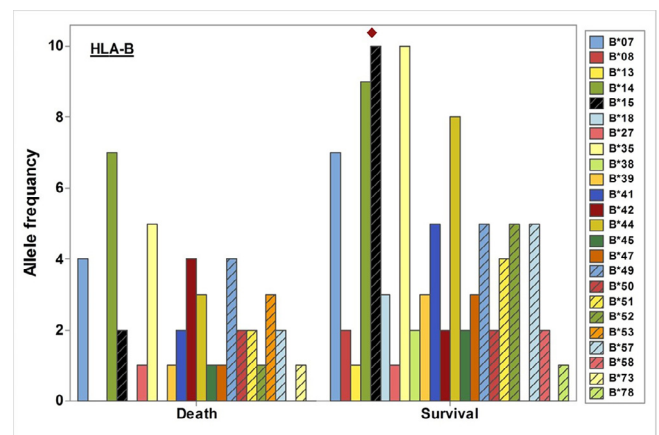
Fig. 2. Frequency of HLA-B and -C alleles in correlation with the mortality from the disease a) Frequency of different HLA-B alleles in correlation with the mortality from COVID-19. a) Frequency of the different HLA-B alleles in correlation with the mortality from COVID-19.
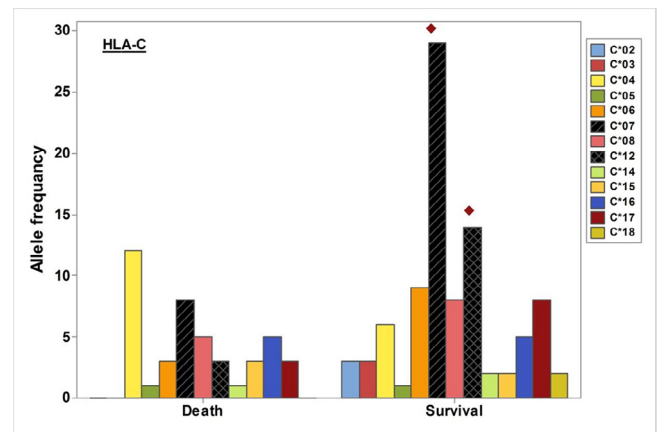
Fig. 2 (continued)

Fig. 1. Frequency of different HLA-B and -C alleles in correlation with the severity of the disease. a) Frequency of the different HLA-B alleles in correlation with the severity of COVID-19. b) Frequency of the different HLA-C alleles in correlation with the severity of COVID-19.

in this cohort. A similarity index of >75% was considered significant for the association. In Fig. 3, HLA-B*41, HLA-C*17, HLA-B*15, and HLA-B*42 formed one cluster with a similarity index of >80%. Another cluster, which included HLA-B*49, HLA-B*44, and HLA-C*16, was also formed.

Table 2
Predictors of survival from COVID-19.

Factors	OR	95% Confidence interval	P
HLA-B*15	1351.06	(4.5021–405445.1879)	**<0.001**
TLC	0.56	(0.3792–0.8196)	**<0.001**
AST	0.93	(0.8481–1.0199)	**0.12**
Ferritin	0.98	(0.9746–0.9943)	**<0.001**
Creatinine	0.36	(0.1733–0.7441)	**<0.001**
IHD (yes)	0.01	(0.0002–0.7158)	**0.03**

Goodness-of-fit test: Hosmer-Lemeshow, χ^2 = 6.14, P = 0.63. P < 0.05 is considered significant.
ALT: alanine aminotransferase, TLC: total leucocytic count, AST: aspartate aminotransferase, IHD: ischemic heart disease.

4. Discussion

Although some studies explored the association between the severity of COVID-19 and HLA genotypes [19,20], little is known about this association in Africa and the Middle East. This study explored this association in Egyptian patients with moderate to severe COVID-19. When patients were stratified by the presence of risk factors and severity of COVID-19, some HLA alleles were significantly correlated with severe COVID-19 pneumonia, whereas the presence of HLA-B*15 was strongly linked with survival. HLA molecules play a multifactorial role in the immune response during viral infections and interact in epitope-dependent or epitope-independent manners to facilitate immune responses [22]. Different HLA genotypes may be associated with a variable T-cell immune response to viral diseases, which could affect the symptoms and outcome of the disease [23]. In general, most viral peptides displayed by major histocompatibility complex-I (MHC-I) molecules on infected cells can induce an immune response [24]. It is useful to have higher binding capabilities of HLA molecules for viral peptides from novel viral infections, such as SARS-CoV-2, on the cell surface of antigen-presenting cells [25]. This higher binding capacity could be associated with better immune response in patients harboring these specific HLA alleles or haplotypes.

HLA-B*15 includes many antigenic specificities that vary in distribution among different human populations [26]. In this cohort, HLA-B*15 was significantly associated with the survival of patients. Moreover, it was the only allele that predicted such survival. Nguyen et al. used bioinformatics to predict the total number of peptides derived from SARS-CoV-2 that a series of HLA class I allelic variants can present. Because these peptides may act as CD8+ T-cell epitopes, they assumed that the more peptides the allele can present, the better is the immune response. Based on their findings, HLA-B*15:03 was among the presenters of the highest number of peptides [27]. They also found that HLA-B*15:03 had the greatest capacity to present highly conserved SARS-CoV-2 peptides commonly shared among different human coronaviruses, suggesting that the presence of this allele could be associated with cross T-cell immunity among different members of these families [27]. Another study used aminopeptidase trimming to determine which peptides of the S1 spike glycoprotein of SARS-CoV-2 can be presented by MHC-I. The findings of the study validated the hypothesis of the previous study. They proved that HLA-B*15:03 was likely to present more SARS-CoV-2 epitopes than HLA-B*46:01, which had the lowest number of predicted binding peptides for the virus [28]. Pretti et al. also found that HLA-B*15:21, HLA-B*15:15, and HLA-B*35:43 had the highest number of strong binders to SARS-CoV-2 peptides among HLA-B alleles [29]. Collectively, our study provides clinical evidence to these results and supports the hypothesis that with more peptides recognized by the HLA allele, the better the T-cell immune response.

HLA-B*15 plays a protective role in other infections as well. A Chinese study found that HLA-B*15 was among the alleles associated with acute self-limited hepatitis and viral clearance in HBV-C2 cases [30]. HLA-B*15 also showed a higher frequency among healthy siblings of families with one or more localized cutaneous leishmaniasis cases in Venezuela [31]. In contrast, two subtypes of HLA-B*15 alleles had an inverse relation with hepatitis C virus (HCV) infection in Chinese blood donors. The frequency of HLA-B*15:01 was significantly higher in the HCV-infected group, whereas HLA-B*15:02 was significantly higher in the uninfected group [32].

In addition to HLA-B*15, HLA-C*07 and HLA-C*12 were associated with protection against mortality, although none of them was predictive of survival. According to Nguyen et al., the HLA-C*12:03 allele was among the top presenters of conserved peptides of SARS-CoV-2, with a high predicted capacity for SARS-CoV-2 epitope presentation [27]. Again, this study adds clinical significance to previous *in silico* results.

In a study that included 82 Chinese patients with COVID-19, HLA-B*15:27 and HLA-C*07:29 were found to occur more frequently in patients than in controls [13]. Two previous studies showed that HLA-C*07- and HLA-C*12-restricted HIV-1-specific cytotoxic T lymphocytes (CTLs) could recognize HIV-1-infected cells and inhibit HIV-1 replication *in vitro* [33,34]. Two studies published later investigated the protective role of HLA-C*12:02 in the context of HIV-1 infection. The first study found that infected persons with both HLA-C*12:02 and the killer cell immunoglobulin-like receptor KIR2LD2 had a lower viral load than those carrying one or not carrying any of them [35], whereas the second study demonstrated that HLA-C*12:02-restricted CTLs specific for two immunodominant epitopes of HIV-1 participate effectively in the suppression of viral replication in infected persons [36].

BK virus is a polyomavirus with high prevalence in the general population but with no significant consequences of infection. However, primary infection or reactivation of this virus in kidney transplant recipients could be associated with allograft dysfunction or loss. Several studies reported that HLA-C*07 might have a protective role from BK virus-associated nephropathy. This protective action could be mediated by a CTL or natural killer (NK) cell immune-mediated response [37–40].

Whereas the aforementioned alleles were protective against mortality, HLA-B*41 and HLA-B*42 were associated with the severe form of COVID-19 in this cohort but with no prediction of mortality. HLA-B*41 was among the most frequent HLA-B alleles in the Egyptian population [41]. HLA-B*41 antigens were among the antigens associated with susceptibility to tuberculosis in Brazilian patients with AIDS [42]. It was also among the alleles associated
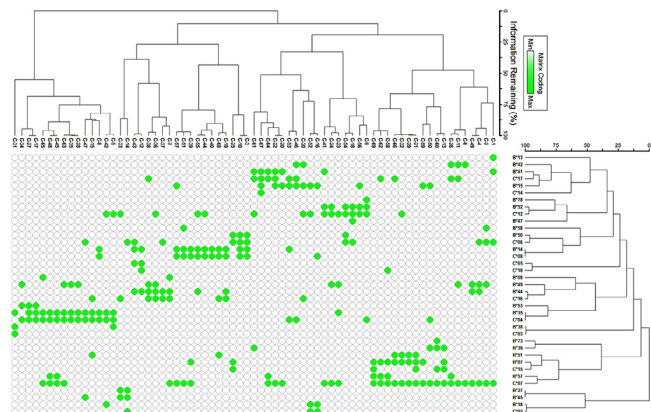
Fig. 3. Two way cluster dendrogram for association between HLA-B and -C alleles. HLA-B*41, HLA-C*17, HLA-B*15, and HLA-B*42 formed one cluster with a similarity index of >80%. HLA-B*49, HLA-B*44, and HLA-C*16 formed another cluster.

with clinical susceptibility to viral persistence and the development of chronic HCV infection in Russian patients [43].

In this cohort, HLA-C*16 and HLA-C*17 were also significantly correlated with severe COVID-19 pneumonia. HLA-C*16 was among the alleles significantly higher in Spanish COVID-19 patients than in healthy controls, but the correlation did not show significance after correction for multiple comparisons [44]. HLA-C*16 was also significantly associated with an increased risk of rapid progression of HIV patients to AIDS [45]. Interestingly, Darke et al. showed a possible association between HLA-B*4101 and HLA-C*1701, where the most common B*4101 haplotype was HLA-A30 or other A allele, C*1701, B*4101, DRB1*1102, or DQB1*0301 and the most common B*4102 haplotype was A*6601 or A3 or other A allele, C*1701, B*4102, DRB1*1303, or DQB1*0301 [46].

During the current pandemic, several studies have been conducted to find which alleles/haplotypes are associated with susceptibility to or protection from infection with COVID-19. An Italian study conducted for this purpose found that the most common haplotype in Italian individuals (HLA-A*01:01 g-B*08:01 g-C*07:01 g-DRB1*03:01) showed a positive correlation (suggestive of susceptibility) with COVID-19. In contrast, the second most common haplotype (HLA-A*02:01 g-B*18:01 g-C*07:01 g-DRB1*11:04) showed a negative correlation (suggestive of protection) from the disease [47].

A Spanish study showed that alleles, such as the HLA-A*11, HLA-C*01, and HLA-DQB1*04, were associated with higher mortality of COVID-19 patients [44]. In contrast, and using bioinformatics tools, a Mexican study found a significant negative correlation between the frequency of HLA-DRB1*01 allele and mortality in hospitalized patients with COIVD-19 [48]. Another *in silico* study showed that HLA-A*11:01 or HLA-A*24:02 genotypes could be associated with the generation of effective T-cell immune responses against SARS-CoV-2 compared to HLA-A*02:01 [49]. Leite et al. also reported that HLA-B*13:01 was protective against the development of COVID-19 daily death rates in Europe, East Asia, and Sub-Saharan Africa [50].

5. Conclusions

This study provides interesting insights into the association between some HLA class I alleles and protection from or severity of COVID-19, which probably occurs through modulation of the immune response. To the best of the authors' knowledge, this is the first study to investigate this relationship in Egypt. These data should be compared to data from other countries to identify persons at risk and understand the difference between countries. Understanding these variations could aid in identifying genetic association with COVID-19, which may predispose certain patients to severe disease forms and/or identify specific targets for personalized treatment. More studies are needed to understand how different HLA class I alleles interact and affect the function of CTLs and NK cells. This entails conducting investigations incorporating epidemiology and genomics with the clinical presentation of COVID-19 patients and assessing these data to perform an evidence-based risk assessment. However, researchers should be cautious while extrapolating these conclusions from regional and mostly limited observations, as it seems that different population haplotypes may be associated with an increased risk of severe disease in different populations.

5.1. Limitations of the study

Despite its promising results, this study has few limitations. First is the small sample size of the patient groups. Second, HLA typing was limited to B and C loci at low resolution, which did not allow the specific subtyping for each of them. However, and as mentioned earlier, this study is the first to investigate the association between HLA class I and the severity of COVID-19 in Egypt. Thus, it can be considered as an exploratory study, and more studies should follow. Future work is needed to confirm these results in other patients and determine whether other HLA loci, such as HLA-DR, HLA-DQ, and HLA-DP, also play a role in the susceptibility to SARS-CoV-2 infection and the development of the severe form of COVID-19.

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.

Author contributions

ASA proposed the idea, coordinated the work, and wrote the first draft of the manuscript. AA analyzed data and wrote the Results section. MF participated in manuscript writing. DMS and MMK supervised data collection and revised the manuscript. LMK and MAK participated in data collection. RMB performed the laboratory analysis and revised the manuscript. All authors have read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2021.09.007.

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