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Impact of HLA-A and B Genotypes on Covid-19 Patients: A Pilot Study among Egyptian Patients

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ABSTRACT

Background: The variation in clinical outcomes among those with severe acute respiratory syndrome caused by coronavirus 2 increased the urgency to identify the pathophysiological characteristics of this disease. Clinical results may be impacted by genes implicated in antiviral defence systems and inflammatory organ damage, particularly the HLA system that is essential for the immunological response.

Objective: Our aim was to determine the HLA-A and -B genotypes of 60 Egyptian COVID-19 cases and connect those findings with illness results, clinical information, and laboratory data.

Patients and methods: A total of 60 Egyptian COVID-19 cases were consecutively recruited from Ain Shams University Hospitals. Only, confirmed cases of SARS-CoV-2 with positive nasopharyngeal swab by real-time RT-PCR were involved in the research. Patients with negative PCR for COVID-19, were neglected.

Results: We found that allele B*41 and A*01 were associated with COVID-19 susceptibility. Whereas allele B*35 was associated with disease severity. The analysis of risk variables using a multivariate regression model revealed that HLA-B*33 was connected to anticipated protection against mortality. Patient survival was affected by increased age, associated comorbidities, high CRP, and elevated serum creatinine.

Conclusion: We concluded that individual HLA class I (A&B) genotypes can affect the association and the severity of COVID-19 and even the protection; through immune response modulation, which might aid physicians in deciding what medical care is most important and greatly reduce COVID-19 mortality.

Keywords: Covid-19, HLA, Genotype, Severity, Association.

INTRODUCTION

The coronavirus illness 2019 (COVID-19) was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was initially discovered in Wuhan, China, at the end of 2019. It quickly spread to Europe, the US, and the rest of the world[(1)]**.** The COVID-19 pathological process exhibits a wide range of clinical signs and symptoms, from undetectable infections to mild (common cold-type), moderate, and even severe (15%) infections; the latter often require hospitalization in ICU to confirm aided breathing support and other medical therapies till wellness or probably the death of the patient. Continuous studies and efforts are ongoing, aiming to determine what really drives the COVID-19 severity and whether it is due to the viral or human factor[(2)]**.**

With the goal of discovering determinants of susceptibility and severity, it is essential to research elements that are significant to host defence. Different immunological responses to a virus in a population may be explained by individual genetic diversity. Variable illnesses severity might result from varied immune response kinetics brought on by polymorphisms in the different innate and adaptive immune response components. Understanding these genetic variations is critical to develop successful treatment strategies[(3)]**.**

HLAs become an important factor in the immune system throughout illnesses when their activities in stimulating or inhibiting immune responses were shown to be critical antigens in transplantation. Individuals with high-risk HLA types might be given priority for vaccination if COVID-19 testing and HLA typing are combined, which could enhance the estimation of the severity of viral illness in the community[(4)]**.** It has been proposed that variations in the number of cases and degree of COVID-19 between various areas of the world may be because of a part to a skewed distribution of HLA alleles related to immune defence against SARS-CoV-2. HLA typing of SARS-CoV-2 infected individuals may assist us in determining alleles participating in susceptibility, protection, and poor prognosis to COVID-19[(5)].

The current study aims to investigate the HLA A &B profiles in a group of Egyptian COVID-19 cases, comparing their (allele frequencies in addition to their associations with the disease severity) with a group of 120 representative controls to identify the predictive value of HLA-genotype on patient's outcome, hoping to help in vaccine modifications and molecular epidemiological research that can contribute to novel therapies.

MATERIAL AND METHODS

Study design

This was a cohort observational study.

Research subjects

A total of 60 Egyptian COVID-19 cases were consecutively recruited from Ain Shams University Hospitals. Only, confirmed cases of SARS-CoV-2

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with positive nasopharyngeal swab by real-time RT-PCR were involved in patient's groups in the research. Patients with negative PCR for COVID-19, were neglected.

The WHO-ISARIC (World Health Organization-International Severe Acute Respiratory and Emerging Infections Consortium) system was used to collect clinical data. COVID-19 disease severity was also assessed via the Sixth Revised Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance.

Each patient had a detailed history taking and clinical assessment. They were also treated based on hospital protocols for distinct illness severity stratifications.

The study population was subdivided into four groups according to COVID-19 severity according to **Wu *et al.* [6]: Group I** included **14** asymptomatic patients, **Group II** included **14** patients presenting with mild symptoms, **Group III** included **15** patients presenting with moderate symptoms, and **Group IV** included **17** patients presenting with severe and critical symptoms.

Demographic data collection

All patients' basic demographics and important clinical parameters, such as age, sex, and history of chronic illnesses, were reported. At the time of hospitalization, laboratory data such as complete blood count (CBC), blood urea nitrogen (BUN), serum levels of albumin, C-reactive protein (CRP), lactate dehydrogenase (LDH), d-dimer, ferritin, and pressure of arterial oxygen (Pa O2) were also collected. Radiology works up (CXR-HRCT of the chest). Respiratory, Cardiovascular, Hepatic and Renal (SOFA score) and Sepsis-induced coagulopathy score (SIC score).

Collection of blood samples

Five millilitres of blood were withdrawn from each patient under complete aseptic condition by venipuncture and collected on a K3EDTA Vacutainer tube (Becton Dickinson, Oxford, UK). The tubes were shaken thoroughly to mix the blood, and they were then stored at −20°C until used for class I HLA -A and B genotyping using the polymerase chain reaction (PCR) Sequence-specific oligonucleotide probes (SSOP) method. Obtained blood samples were not used in any other study. If the stored samples will be used for other objectives, new informed consent will be obtained from the participants.

HLA-A & B genotyping

Total genomic DNA was extracted, and HLA-A and B alleles were molecularly typed by polymerase chain reaction (PCR), followed by reverse hybridization with a panel of sequence-specific oligonucleotide probes (reverse SSOP). The alleles were assigned depending on the hybridization behaviour for several probes utilizing the LiRAS™ for LiPA HLA v7.01 CE software interpretation.

DNA extraction QIAamp DNA Mini Kit (Qiagene, USA) was developed to quickly purify an average of 6µg of total DNA from 200µl of human blood in EDTA. The Nanodrop device was used to profile all DNA samples for concentration and purity; the purity of the samples ranged from 1.72 to 1.93 to be enrolled for HLA-typing.

HLA-A and B amplification

The INNO-LiPA HLA-A and B Amplification Plus Kits (Fujirebio, Belgium; Code-Key: FRI92425) were used to amplify nucleic acid of exons 2 &3 of the HLA-A and B locus carried out by PCR. INNO-LiPA HLA-A and B Plus Is a line probe assay (Fujirebio, Belgium; Code-Key: FRI86640 for HLA-A and FRI47554 for HLA-B), designed for the molecular typing of human leukocyte antigen (HLA) A and B alleles at the allele group level.

Ethical consent

After describing the purpose and procedures of the study and assuring data confidentiality, study participants provided informed consent. The research was performed with the permission of the Ain Shams University Ethics Committee (FWA00017585). The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduct of this study.

Statistical analysis

Statistical analyses were carried out utilizing IBM-SPSS Statistics Version 26 (IBM SPSS Japan, Tokyo, Japan). The Shapiro Walk test was used to determine whether the data distribution was normal. Frequencies and relative percentages were used to depict qualitative data. To assess differences between two or more sets of qualitative variables, utilise the chi square test (2). Standard deviation (SD) was used to express quantitative data as mean. To compare two independent groups of regularly distributed variables (parametric data), the independent samples t-test was employed. P values lower than 0.05 were regarded as significant.

RESULTS

The study included 60 patients diagnosed with Covid-19 infection depending on clinical assessment, radiological findings, and SARS-CoV-2 real-time PCR. The frequency of HLA-A &B alleles genotyping was fitted for the Hardy-Weinberg equation after considering the Pc-value among 120 Egyptian subjects.

Among the studied genotypes, HLA **A*01** allele frequency was markedly higher among the COVID-19 group in comparison to the control (p value:0.002). Both groups were comparable regarding the expression of HLA-A* **02, 03, 26, 30** and **33** alleles. HLA B***41** allele was more frequent among the COVID-19 group than the control group, with a statistically significant difference (p-value: 0.04) **(Table 1).**

Table (1): Comparison of HLA alleles frequencies among covid-19 patients and control groups

Alleles	Covid-19 group (60 cases) No. (%)	Control group (120) No. (%)	95% confidence interval		Odds ratio (%)	P value
			Lower	Upper		
HLA-A						
1	26 (43.33%)	25 (20.83%)	1.19	4.67	2.9	***0.002***
2	26 (43.33%)	46 (38.33%)	0.65	2.3	1.23	0.15
3	8 (13.33%)	14 (11.67%)	0.45	2.95	1.16	0.75
26	10 (16.6%)	12 (10%)	0.73	4.44	1.8	0.2
30	13 (21.67%)	23 (19.17%)	0.54	2.5	1.16	0.69
33	9 (15%)	19 (15.83%)	0.39	2.22	0.93	0.88
HLA-B						
14	14 (23.33%)	23 (19.17%)	0.6	2.7	1.28	0.5
35	16 (26.67%)	23 (19.17%)	0.73	3.18	1.53	0.25
38	11 (18.33%)	14 (11.67%)	0.72	4.01	1.69	0.23
41	12 (20%)	11 (9.17%)	1.022	6.007	2.47	***0.04***
44	9 (15%)	11 (9.17%)	0.68	4.48	1.7	0.24
49	7 (11.67%)	17 (14.17%)	0.31	2.04	0.8	0.64

Laboratory investigations including complete blood counts and liver enzymes did not affect disease severity and did not show a difference of statistical significance between the 4 groups; however, CRP was significantly higher in cases with severe disease (p-value: 0.03). Renal insufficiency reflected by increased serum creatinine at the time of Covid-19 diagnosis is considered a risk factor or marker for disease severity of statistical significance (p-value: 0.04) **(Table 2).**

Table (2): Comparison of Clinical and laboratory investigations at the time of presentation among the four groups

	Mild disease (14 cases)	Moderate disease (14 cases)	Severe disease (15 cases)	Critically ill (17cases)	P value
CORAD score: 1 2-3 4-5	 14 (100%) 0 0	 11 (78.5%) 2 (14.2%) 1 (7.1%)	 4 (26.7%) 3 (20%) 8 (53.3%)	 8 (45%) 0 9 (52.9%)	***0.0001***
Hemoglobin (g/dL) mean ±Sd	11.15±2.6	10.96±2.5	11.12±2.6	10.18±1.68	0.625
White blood cells (/mm^3) mean ±Sd	7.3±1.8	7.26±1.7	9.7±2.3	9.1±2.2	0.496
Lymphocytes (/mm^3) mean ±Sd	1.6±0.4	1.3±0.3	1.1±0.2	1.2±0.2	0.326
Platelets (/mm^3) mean ±Sd	249.0±60.6	217.5±52.4	266.6±64.2	259.12±62.4	0.541
ALT (iu/L) mean ±Sd	24.07±5.8	39.5±9.1	71.27±17.7	69.7±11.1	0.372
AST (iu/L) mean ±Sd	35.14±8.6	38.64±9.5	35.2±8.2	55.35±13.6	0.315
D-dimer Mean ±Sd	0.64±0.14	0.92±0.22	34.47±8.4	1.6±0.3	0.3
S. ferritin Mean ±Sd	210.8±51.7	218.69±22.2	229.12±50.6	204.89±40.3	0.07
CRP Mean ±Sd	56.77±13.6	13.36±3.1	54.11±13.3	66.53±16.4	***0.03***
S. creatinine (mg/dL) mean ±Sd	0.9±0.22	1.02±0.24	0.95±0.22	2.3±0.54	***0.04***

The HLA typing of each group was studied to find a correlation between HLA allele types and disease severity.

The 4 groups were comparable regarding HLA-A genotyping. Studying HLA-B typing showed that HLA **B*35** had higher frequency among severe and critically ill groups with statistically significance (p-value: 0.006). On the other hand, HLA **B*44** was more frequent among the mild disease group, and HLA **B*49** was more frequent among the moderate disease group with a statistically significant difference (p-value: 0.027, 0.04 resp.).

HLA **B*35** and HLA **B*41** affected disease severity in different ways; patients with more severe disease had increased frequencies of HLA **B*35**, whereas the presence of HLA-**B*41** is considered protective against severe infection (p-value: 0.04). For HLA-A **A*02** allele was more frequent among the group with mild clinical symptoms (p-value:0.09) **(Table 3).**

From our data, we found that increased age, associated comorbidities, high CRP, and elevated serum creatinine, are considered risk factors for increased disease severity; alongside with presence of HLA typing HLA **B*35** in spite that HLA **B*41** expression was associated with less chance to develop severe symptoms. These factors were applied to the multivariate model to analyze risk factors for more accurate determination.

Age, diabetes, and renal insufficiency kept their values as risk factors for disease severity other than HLA results. HLA **B*35** is still a significant risk factor (p-value: 0.003), while HLA **B*41** lost its significance as a protective factor against severe disease. Another HLA allele appeared to be protective as HLA **A*33** (p-value: 0.039) (R Square: 0.614, adjusted R Square: 0.384, Significant F changes: 0.004, Regression p-value: 0.004).

Table (3): Comparison of HLA typing and disease severity

	Mild disease (14 cases)	**Moderate disease (14 cases)**	**Severe disease (15 cases)**	**Critically ill (17cases)**	**P value**
HLA-A No. (%)					
1	5 (35.7%)	6 (42.8%)	8 (53.3%)	7 (41.17%)	0.97
2	10(71.4%)	6 (42.8%)	5 (33.3%)	5 (29.4%)	***0.09***
3	1 (7.1%)	1 (7.14%)	3 (20%)	3 (17.65%)	0.62
26	2 (14.2%)	2 (14.2%)	3 (20%)	3 (17.65%)	0.25
30	2 (14.2%)	5 (35.7%)	2 (13.3%)	4 (23.5%)	0.44
33	4 (28.4%)	1 (7.14%)	2 (13.3%)	2 (11.76%)	0.47
HLA-B No. (%)					
14	3 (21.4%)	3 (21.4%)	4 (26.7%)	4 (23.5%)	0.98
35	2 (14.2%)	1 (7.14%)	9 (60%)	4 (23.5%)	***0.006***
38	2 (14.2%)	3 (21.4%)	3 (20%)	3 (17.6%)	0.94
41	3 (21.4%)	4 (28.6%)	2 (13.3%)	3 (17.6%)	0.77
44	6 (42.8%)	0	1 (6.67%)	2 (11.76%)	***0.027***
49	0	5 (35.7%)	1 (6.67%)	1 (5.9%)	***0.04***

DISCUSSION

The scientific community is intensely concentrating on figuring out the characteristics of the immune response to the Covid19 virus and how heredity affects illness susceptibility and severity. An international partnership of European centres was set up to examine the issue of whether there were any possible genetic host variables related to the severe clinical development of the SARS-CoV-2 infection during the outset of the COVID-19 pandemic in Europe in the spring of 2020[7]**.** HLA genes are significant factors in an individual's response to a foreign pathogen. Therefore, in this investigation, we were interested in finding HLA-A and B alleles that are vulnerable and those that may be employed in risk prediction models for the early detection of severe COVID-19 in COVID-19 cases who are hospitalized.

Migliorini *et al.* [5], when investigating the connection between COVID-19 susceptibility and HLA genotypes, they argued that while genetic and other environmental factors may have an effect, the presence of HLA and other polymorphisms increased the patient's vulnerability**. Douillard *et al.* [1]** and **Tavasolian *et al.* [6]** also reported a similar outcome.

We identified that HLA **A*01** and HLA **B*41** alleles were more common among Covid-19 patients with a statistical difference (p-value of 0.002 and 0.04, respectively). However, HLA **B*35** were infrequent among patients diagnosed with Covid-19. **Lorente** and his team in Spain provided information on COVID-19 patient HLA genetic variants, susceptibility to, and prognosis. They discovered a tendency for alleles HLA-A*32 to be more prevalent in healthy controls than in COVID-19 patients, as well as alleles HLA-A*03, HLA-B*39, and HLA-C*16 to be more prevalent in COVID-19 cases than in healthy controls[8].

Langton *et al.* [3], in their study, noted that the Covid-19 severity of infection might be linked to the patient's genetic susceptibility. Our data showed that HLA **B*35** had higher frequency among severe and critically ill groups with statistically significance (p-value: 0.006). On the other hand, HLA **B*44** was more frequent among the mild disease group, and HLA **B*49** was more frequent among the moderate disease group with a statistically significant difference (p-value: 0.027, 0.04 resp.).The authors hypothesized that poor linking of viral peptides to HLA B*22 alleles might lead to a decreased T cell response despite the fact that B22+ individuals did not have the further serious disease than individuals with other HLA alleles. A strong positive correlation between the HLA B*22 serotype and SARS-CoV-2 infected Chinese individuals was noticed[9].

In their multi-variate study, **Norin *et al.* [10]** found that black COVID-19 patients who were hospitalised and HLA B*53 positive had a 7.4-fold higher chance of death than black COVID-19 individuals who were B*53 negative. With the exception of a little area at the 30 end of exon 2, HLA B*53 and HLA B*35 are substantially similar because of their tight structural relationship. As a result, the two alleles exhibit homology at the class 1 heavy chain's alpha 2 and 3 domains but vary in the alpha 1 domain by five amino acids, notably at residues 77, 80-83[11]. This explanation makes their results near to ours. In Iran**, Alnaqbi *et al.* [12]** found that the genotypes HLA-A*03:01 and supertype B*44 substantially correlated with the severity of the condition, whereas the alleles HLA-B*51:01 and HLA-A*26:01 were considerably greater in the non-hospitalized individuals, indicating a protective connection. **Wang *et al.* [13]** in their research, noted that **B*15:27** alleles can be linked to the incidence of COVID-19.

On the contrary, results obtained in a study carried out by **Warren and Birol[14]** revealed that in the COVID-19 group, the allele did not seem to put them at risk of hospitalization. The Covid-19 illness severity outcome inspection shows nominally significant risks linked to **A*11:01** and **DQA1*01:02**. It is crucial to investigate HLA genes in many ethnic groups in order to comprehend the genetics of the disease since these conflicting results might be caused by differences in ethnicity.

Iturrieta-Zuazo *et al.* [15] demonstrated a larger proportion of HLA homozygosity in Spanish patients with a severe infection in locus A and C when compared to moderate and mild patients, suggesting that the level of HLA homozygosity may influence the severity of the SARS-CoV-2 infection.

Limitations of our work, because of the small sample size, for investigating the impact of COVID-19 susceptibility and outcomes for patients caused by HLA gene variants which highlight the importance of continuing to survey with a larger sample size for the purpose of identifying HLA alleles in both class I & II to increase our comprehension of the genetic foundation for Covid-19, In order to ensure new treatments might be created to avoid or reduce its severity. Also, we studied clinical disease severity at a limited period, and clinical follow-up at a different time have not been researched. One explanation for the lack of this research in our country may be the challenge of gathering accurate phenotypic data on the reaction or immune response to the virus using typical medical data or electronic health records.

CONCLUSIONS

Our early pilot investigation revealed that HLA genetic variants may be connected to infectivity with regard to the severity of COVID-19. In this investigation, we demonstrated an association between the COVID-19 susceptibility alleles B*41 and A*01 in Egyptian patients. Whereas allele **B*35** was associated with disease severity. However, further research with a bigger sample size is necessary before any firm conclusions can be made.

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